PATHCOM SYSTEMS CORPORATION

BIOCARE MEDICAL

ONCORE™ Pro X

Specialist User Manual

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RUO

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Instrument Information

Oncore Pro X Slide Stainer Model: ONCPRX0001
Software Version: 4.0

Computer Configuration:
Laptop or Desktop Computer with Mouse
Instrument Communications Interface: USB 2.0
USB Cable must be <3 meters (10 feet)

The use of this instrument is fully licensed under U.S. Patent no. 5,839,091; U.S. Patent no. 7,476,543; U.S. Patent no. 7,635,453; and U.S. Patent no. 7,977,086 B2.

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Section 1: Introduction

The proper development of protocols and reagents is essential to the effective performance of an automated slide staining instrument. In order to meet the specific needs of a diverse range of users and staining applications, the Oncore Pro X Slide Stainer provides a flexible protocol development workflow via three levels of protocol customization: creation of base protocol templates, fine-adjustment of key steps in standard protocols, and full step-by-step customization of special protocols.

Each protocol may be fine-tuned to the level required by the staining application. In addition, users may adjust reagent handling based on the viscosity properties, hazardous classification and on-board reagent mixing requirements. Finally, protocols and templates may be easily exchanged between end-users and collaborators using the built-in import/export tools.

This manual provides a guide to aid staining application specialists in the development and optimization of protocols using the Oncore Pro X Slide Stainer system.

Figure 1

Key Terms

1) Protocol: a sequence of steps (reagent incubation steps and wash steps) used to execute a staining run.
2) Protocol Template: a protocol used as a base to generate other protocols.
3) Standard Protocol: a protocol generated from a protocol template.
4) Special Protocol: a custom protocol.
5) Protocol Type/Reagent Type: a category defined by the staining application or detection system.

General Recommendations

a. Use serial sections of verified control tissue
   - Verify that samples received proper fixation/pretreatment.
   - Verify that samples are positive for the marker of interest.
   - Place sections in the recommended staining area, avoiding the injection site.

b. Verify instrument function
   - Verify reference point calibrations
   - Clean chambers and inspect regularly for cracking/damage
   - Inspect and clean tubing regularly
   - Verify pump function
   - Stain control slides with an antibody that generates a strong positive reaction under default conditions, such as CD20, CD3, CD79a, or CD8, etc.

c. Prepare common reagent solutions following the vialing guidelines
   - Verify sufficient volume/number of tests, account for dead volume and evaporation
   - Verify stability at RT and expiration date

d. Prepare serial dilutions of the antibody as needed
   - Use the manufacturer's recommended dilution to start (ie: 1:100 or RTU)

e. Perform an initial test using the manufacturer's default protocol conditions
   - If a default protocol is not available, check the antibody testing database for recommended staining conditions.
   - If staining is satisfactory under initial test conditions, no further adjustment is needed; perform repeatability with >3 slides and/or different tissue types.
   - If staining is not satisfactory, further adjustment is needed based on the results, which may involve adjusting protocol conditions, changing the primary antibody dilution/clone, changing to a different template, or creating special protocols, etc.
   - Further fine-tuning may be required at the end-user site, such as reducing TR conditions to improve morphology, decreasing antibody concentrations to reduce background and costs, etc.

Note: The system's default protocol templates have been developed in coordination with the manufacturer's specially formulated reagent kits with consideration to balancing the staining intensity, background, uniformity, and consistency, while minimizing slide-to-slide variation and overall running time. The proper optimization of new staining protocols may require adjusting the compositions and concentrations of the key reagents. It is vital to establish a complete and robust staining system to provide a strong foundation for protocol development, thereby reducing the time spent on extensive troubleshooting and optimization at the end-user level.

Section 2: Creating and Customizing Protocols

The PCStainer's Protocol Editor provides specialists the basic tools to add/remove/edit protocols, assign protocol templates, and fine -tune key protocol steps. *Refer to the Operating Manual for further details.*

Click  and login to the application using a Distributor-level account.
Default User ID: Distributor
Default Password: Distributor

Protocols may be classified into 3 categories: protocols, protocol templates and special protocols. To avoid confusion, this document will reference any protocol generated from a template as a "standard" protocol.

2.1 Standard Protocols

Overview of Standard Protocols
A standard protocol is a protocol generated from a protocol template.

Standard protocols are grouped by Protocol Type, referring to the staining application or detection system.
For routine staining, users should implement a set of standard protocols generated from the same/similar protocol templates to maximize runtime efficiency.

Examples of Standard Protocol Types

a. HRP: protocols for 2-step HRP Polymer -DAB detection
b. HRP Plus: protocols for 3-step HRP Polymer -DAB detection
c. HRP Super: protocols for double retrieval 3-step HRP Polymer -DAB detection
d. HRPx: protocols for 3-step HRP Polymer -DAB detection (enhanced)
e. HRPx: Plus: protocols for double retrieval 3-step HRP Polymer -DAB detection (enhanced)
f. AP: protocols for AP Polymer -Red detection
g. AP Plus: protocols for 2-step AP Polymer -Red detection
h. APx: protocols for 2-step AP Polymer -Red detection (enhanced)
i. APx Plus: protocols for double retrieval 2-step AP Polymer -Red detection (enhanced)
j. Double Stain: protocols for double staining (HRP+AP) applications
k. ISH HRP Super: protocols for in situ hybridization with 2-Step HRP Polymer -DAB detection
l. ISH AP Super: protocols for in situ hybridization with 2-Step AP Polymer -Red detection
m. Cyto: protocols for cytological/frozen tissue section samples
n. FISH and FISH Plus: protocols for fluorescence in situ hybridization
o. CISH: protocols for chromogenic in situ hybridization

The full list of protocols assigned under each Protocol Type is available in the System Utilities>Editors>Protocol Editor. Select the tab of the appropriate Protocol Type to view the protocols.

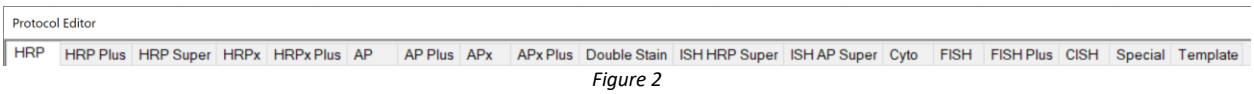


Figure 2

Creating a New Standard Protocol

The user may add more standard protocols to add different antibody and probe products to the system, distinguish different clones/titers/reagent vendors, and to implement different customization options.

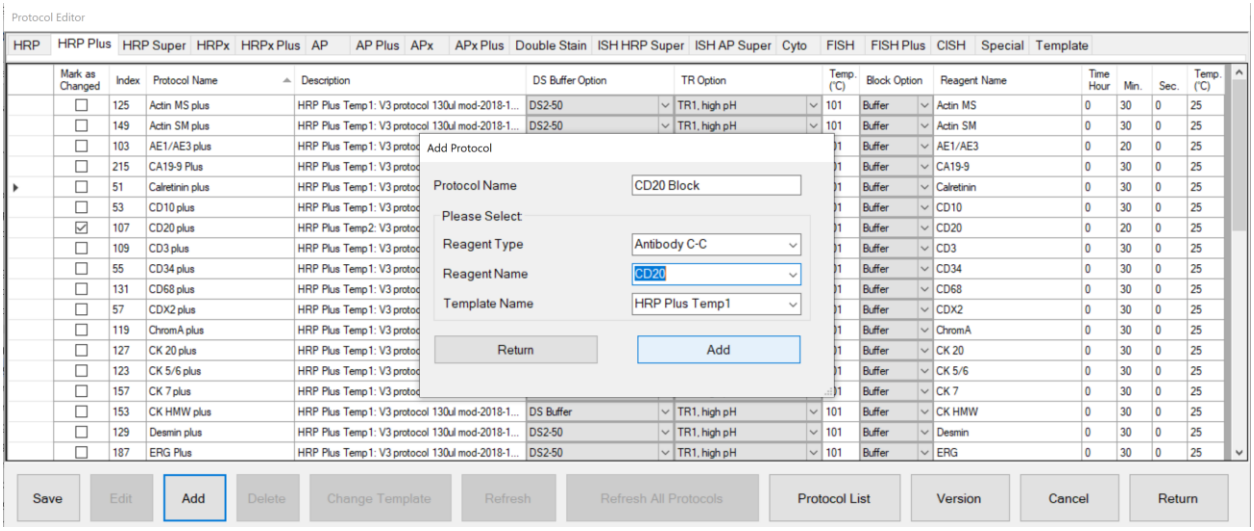


Figure 3

To add a new protocol, open System Utilities>Editors>**Protocol Editor**.

a. Select the appropriate Protocol Type tab: HRP, AP,…etc.
b. Click "**Add**".
c. Enter the Protocol Name.
   - *Note 1: Each protocol must have a unique protocol name. Append a short suffix to the name to distinguish protocols with the same primary reagent.*
   - *Note 2: To avoid conflicts with positive or negative controls in the programming software, please refrain from using the symbols "+" and "-" at the end of the protocol name.*
d. Select the Reagent type and Reagent Name of the primary reagent.
e. Select the Template Name.
f. Click "**Add**" to generate the new protocol from the selected protocol template.

Deleting a Standard Protocol

Deletion of protocols may be restricted based on the user's security level. *Refer to Security for more information.*

To delete a protocol, open System Utilities>Editors>**Protocol Editor**.

a. Select the protocol using the grid in the left-most column, then click "**Delete**".
   - *Note: Protocols must be deleted one at a time.*

Modifying a Standard IHC Protocol

Each standard protocol may be fine-tuned using several built-in customization options in the main Protocol Editor. *Refer to Protocol Customizations for more details.*

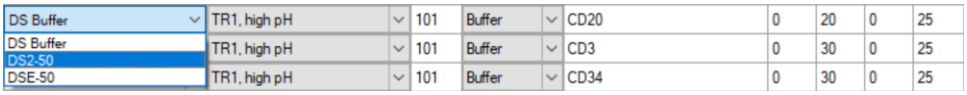


Figure 4

To modify an IHC protocol, open System Utilities>Editors>**Protocol Editor**.

a. Select the appropriate Protocol Type tab: HRP, HRP Super, AP, AP Super, …etc.
b. Locate the protocol in the list.
c. Select a DS Buffer Option (pretreatment after deparaffinization) DS Buffer, DS2-50, DSE-50.
d. Select a TR Option (target retrieval/heat-induced epitope retrieval): TR1, TR2, TR3, TR4, TR Buffer, TR Enzyme.
e. Set the TR temperature: Range limit between RT and 110C.
f. Select a Block Option (H2O2 Blocking step after TR): Buffer, Block.
g. Set the Ab Incubation Time. Range limit between 5-120 minutes.
   - *Note: The Heat Incubation Time is automatically set to equal to the Ab Incubation Time.*
h. Set the Ab Incubation Temperature. Range limit between RT and 110C.
i. Click "**Save**" to save all changes to the protocol.

Modifying a Standard ISH Protocol

Each standard FISH protocol may be fine-tuned using the built-in options available in the Protocol Editor.

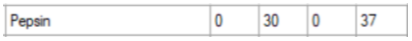


Figure 5

To modify an ISH protocol, open System Utilities>Editors>**Protocol Editor**.

a. Select the appropriate Protocol Type tab: FISH, FISH Plus, ISH HRP Super, ISH AP Super, etc.
b. Locate the protocol in the list.
c. Set the enzyme digestion time.
d. Set the enzyme digestion temperature.
e. Click "**Save**" to save all changes to the protocol.

Saving Protocol Customizations

The user can save a set of all current protocol modifications (under all Protocol Type tabs) for later use/recovery. This will enable the system to restore customizations after refreshing or changing protocol templates.

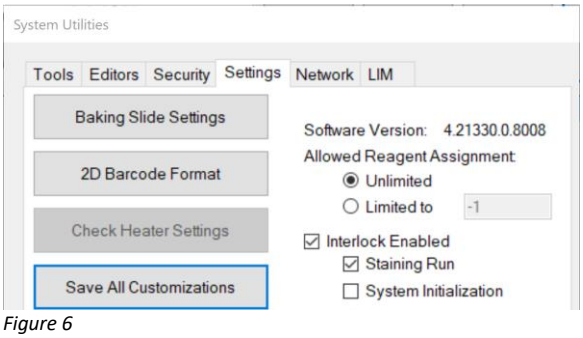


Figure 6

To save all current protocol customizations, open System Utilities>**Settings.**

a. Click "**Save All Customizations**".
   - *Note: The original set of saved customizations (for all Protocol Types) will be overwritten.*
b. *Tip: Before saving all customizations, make a backup of the C:\PathCom folder to use as a restore point.*

Refreshing Protocols to Restore Customizations

The user can revert protocols back to the previously saved customization or the manufacturer's default to recover from unwanted changes/mistakes.

To refresh one or more protocols, open System Utilities>Editors>**Protocol Editor**.

a. Select the protocol(s) using the grid in the left-most column, then click "**Refresh**".
b. Select the option to "Refresh with saved customization" or "Refresh with manufacturer default".

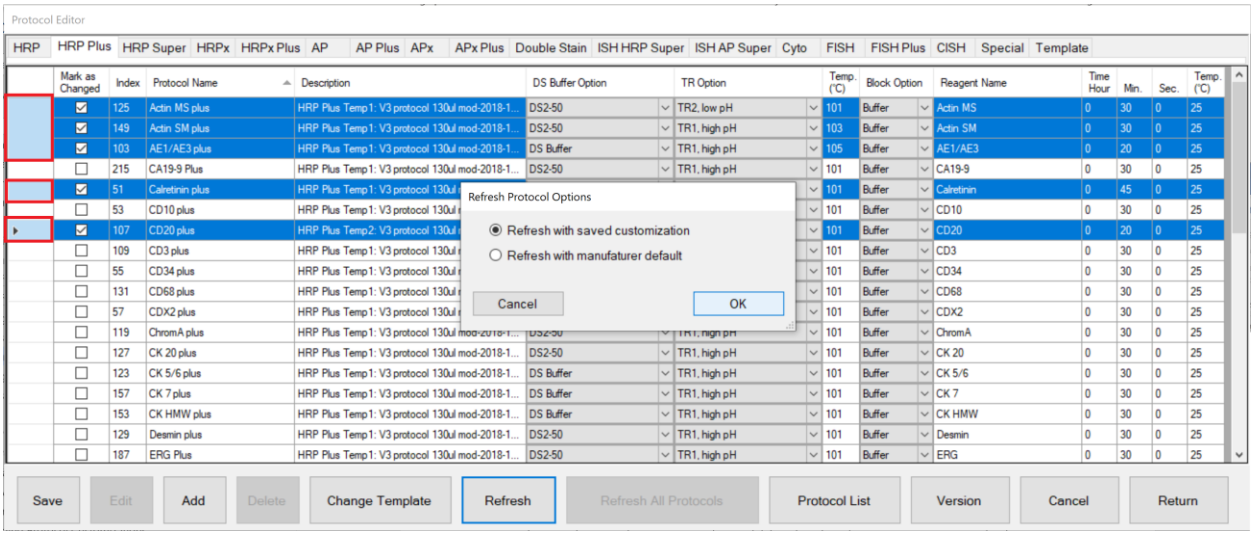


Figure 7

To refresh all protocols within the Protocol Type tab, open System Utilities>Editors>**Protocol Editor**.

a. Select all protocols using the upper-most left corner grid, then click "**Refresh**".
b. Select the option to "Refresh with saved customization" or "Refresh with manufacturer default".

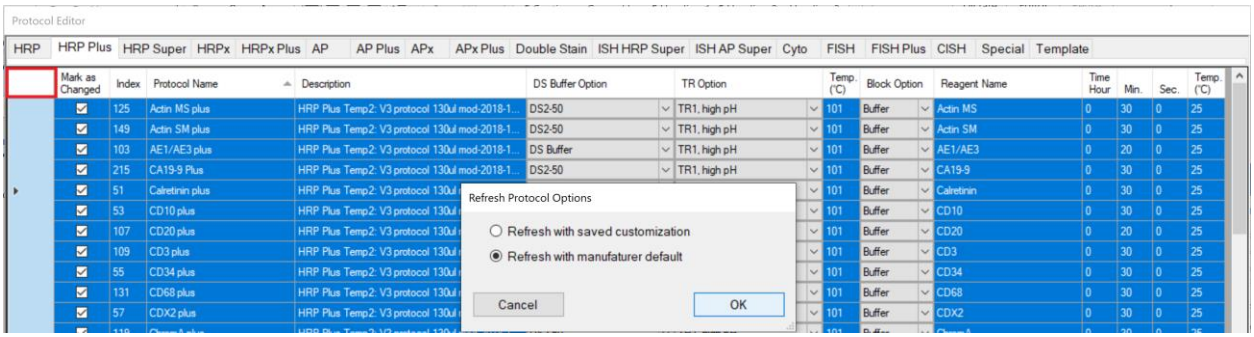


Figure 8

Changing the Assigned Protocol Template

The user may change the assigned protocol template used to generate each standard protocol.

To change the protocol template for one or more protocols, open System Utilities>Editors>**Protocol Editor**.

a. Select the protocol(s) using the grid in the left-most column, then click "**Change Protocol Template**".
b. Select a new template and click "**OK**".
c. The software will automatically regenerate the protocols using the selected template and apply the saved protocol customizations.
d. Note: The user may create subsets of protocols within the same Protocol Type, that are generated from different protocol templates to accommodate different pretreatment, retrieval, and counterstain options, etc.

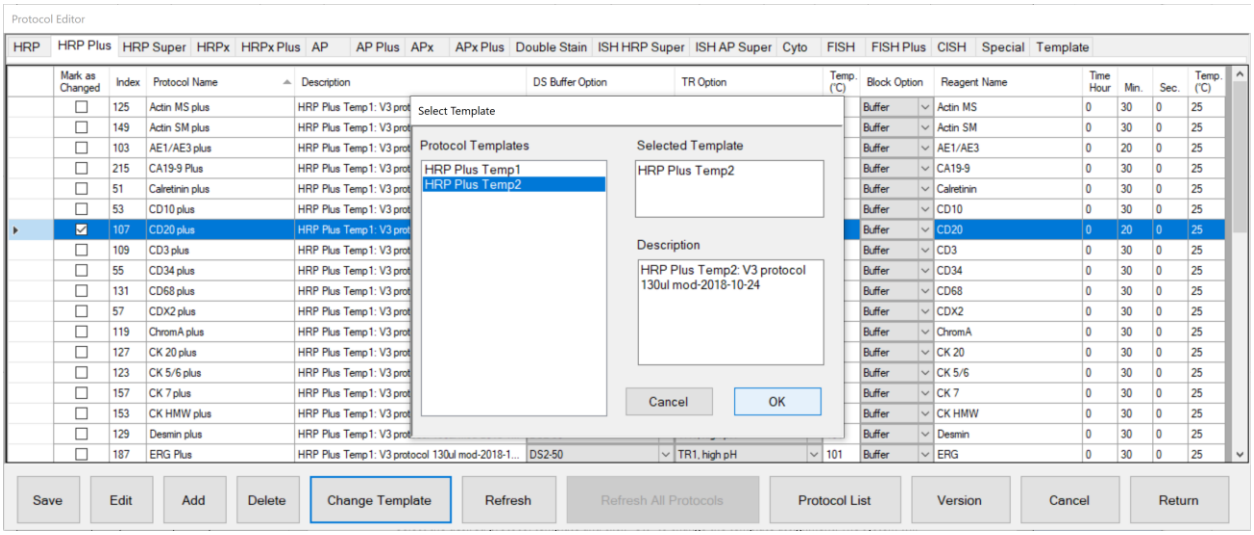


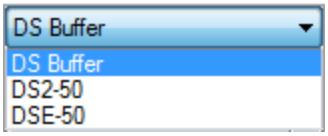
Figure 9

Protocol Customizations

The user may customize key protocol steps directly in the main Protocol Editor.
Refer to Appendix I: Protocol Special Requirements and Recommendations for more details.

IHC: Pretreatment Option

The pretreatment step functions to remove remaining wax residue, provide tissue pretreatment, and preheat the slide before the target retrieval step. Default incubation time 4m30s at 58C.
Note: The name of the option reagents may vary based on the system configuration.

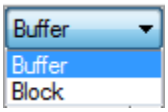


Change DS2 options:

1) DS2-50: Pretreatment solution
   a. Certain stains may show enhanced staining intensity when the tissue is pretreated with DS2.
   b. Examples: MLH1, MSH2, MUM1, ZAP-70
2) DS Buffer: System Fluid (default)
   a. Certain stains may be sensitive to the chemical components in DS2; substitute with DS Buffer.
   b. Examples: P170, C-erbB-2, CD20, CD10 on some colon
   c. Examples: CD138, ChromA
3) DSE-50: Pepsin, or substitute with user's enzyme of choice, protease K, Pronase, etc.
   a. Certain stains may require enzyme digestion of the tissue before the heated target retrieval step.
   b. Examples: Rb Gene, Inhibin
   c. *Note: It may be necessary to change the template to optimize the enzyme incubation and/or adjust the enzyme dilution. DS Enzyme 39C for 8'.*

IHC: Peroxidase Block Option

The hydrogen peroxide block step functions as a "chemical block" to inactivate endogenous peroxidase activity before the target retrieval step.
Note: The name of the option reagents may vary based on the system configuration.

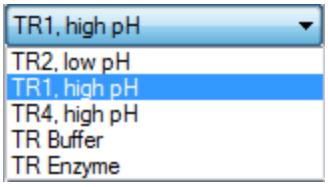


Change Block options:

1) Block: Hydrogen Peroxide solution
   a. Note about endogenous peroxidase block: The high temperature target retrieval step will perform a "heating block" function to inactivate endogenous peroxidases. TR1 and TR4 solutions will additionally perform a "chemical block" function. TR2 does not perform a "chemical block" function. Therefore, if there are concerns about insufficient blocking on certain tissues, such as bone marrow, use Block for TR2 protocols, TR Enzyme and TR Buffer protocols.
2) Buffer: System Fluid (default)

IHC: Target Retrieval Option and Temperature

The target retrieval step functions to expose the desired antigen/target for subsequent binding to the antibody. Default incubation time 27 minutes (21 min heat up at 101C, with 6 minute cool-down to 50C).
Note: The name of the option reagents may vary based on the system configuration.



Change the TR options:

1) TR1: High pH 9 Target Retrieval solution (default)
2) TR2: Low pH 6 Target Retrieval solution
a. Certain stains may show better results using low pH TR2 solution.
b. Examples: CD138, D2-40
c. Examples: CD45RO, CD44, CK pan
3) TR4: Enhanced High pH Target Retrieval solution
    a. Certain difficult stains may show better results using TR4 solution.
    b. Examples: ER, PR
4) TR Buffer: System Fluid. Set temperature to 37C.
    a. Certain stains do not require heat-mediated retrieval. *The system lists this option by default.*
    b. Examples: HBcAg
5) TR Enzyme: User's enzyme of choice. Set temperature to 37C.
    a. Certain stains will only require enzyme retrieval. *The system lists this option by default.*
    b. Examples: EGFR/37c for 32'
    c. *Note: It may be necessary to change the template to optimize the enzyme incubation, and/or adjust the enzyme dilution. Default TR volume is 370uL with agitation cycle 13 minutes at A3/14 minutes at A4.*
    d. IMPORTANT! Temperature must be reduced to prevent excessive evaporation.
6) Add additional TR options. *Note: TR3, ISH: Retrieval solution for FISH and CISH is restricted from this list.*
    a. Open System Utilities>Editors>Reagent Editor
    b. Add a new TR reagent under the Reagent Type, "Retrieval".

Change the TR temperature:

1) 101C (default)
2) Lower TR temperature, 98/99C
    a. Certain stains may show better results at lower temperatures
    b. Examples: CD20/98c is better than 101c.
    c. Certain fresh tissues, 99c will have better results than 101C.
    d. Note: Generally, decreasing temperature will decrease staining intensity.
3) Higher TR temperature, 103C (maximum)
    a. Certain stains may show better results at higher temperatures
    b. Examples: Cyclin D1/102c; Pax-5/104c; p504s/104c; Galectin 3/104c; D2-40
    c. Examples: MLH1, Villin; BCL-2
    d. Note: Generally, increasing temperature will increase staining intensity.
4) 37C or RT/25C for use with TR Enzyme and TR Block, TR Buffer
    a. Certain stains do not require heat-mediated retrieval
    b. Examples: HBcAg, Actin SM

Tip: Although it is possible to set any temperature between 25C-103C, it is important to limit the number of different temperature set points to improve runtime efficiency. Only identical steps (same reagent, incubation time, temperature) may be pooled and run together.

IHC: Antibody Incubation Time and Temperature

The antibody incubation step functions to bind the primary antibody to its target for subsequent detection.

Time Hour	Min.	Sec.	Temp. (°C)
0	30	0	25

Change the primary antibody incubation time:

1) 30 minutes (default). Incubation time should range between minimum 10 minutes and maximum 60 minutes.
2) Most antibodies require a minimum incubation time of 15-20 minutes.
3) Certain antibodies may require a longer incubation time of 45-60 minutes to increase the staining intensity.
    a. *Note: Longer incubation times may result in significant loss of staining area due to evaporation. Therefore, it may be more effective to optimize the retrieval conditions or change to a stronger detection system. Users may also need to optimize the antibody concentrations or choose a different clone to achieve a good balance on intensity, staining area, background/non-specific binding and incubation time.*

Change the primary antibody incubation temperature:

1) RT/25C (default)
2) Most antibodies work well within the temperature range of 25-37C.
    a. It is not recommended to set a temperature lower than RT.
3) Certain antibodies may work better at RT than at 37C.
    a. Examples: ER (1D5), CD45/LCA
4) Certain antibodies may work better at 37C than at RT.

ISH: Enzyme Incubation Time and Temperature

The enzyme digestion step functions as a pretreatment step before probe hybridization.

Time Hour	Min.	Sec.	Temp. (°C)
0	30	0	37

Change the enzyme incubation time:

1) 30 minutes (default). Time varies, depending on the enzyme and probe application.
    a. Note: Enzyme formulation may require adjustment to minimize variation between slides.

Change the enzyme incubation temperature:

1) Generally, 37C or below

Managing Protocol Customizations

The user should regularly save and backup all protocols, reagents and customizations to prevent accidental loss of data due to PC failures, file corruption, accidental overwriting and user error.

a. Save Reagents

System Utilities>Editors>Reagent Editor: "Save"

The reagent is automatically saved when it is added to the system.

b. Save Protocols

System Utilities>Editors>Protocol Editor: "Save"

The protocol must be saved after modifying the protocol customizations.

c. Save All Customizations

System Utilities>Settings: "Save All Customizations"

The protocol customizations must be saved to create a restore point ie: after validating the staining protocols.

d. Restore Saved Customizations

System Utilities>Editors>Protocol Editor: "Refresh Protocol with Customizations"

The protocols can be restored to previous staining conditions using saved customizations (from restore point).

e. Restore Default Customizations

System Utilities>Editors>Protocol Editor: "Go Back to Manufacturer Default"

The protocols can be restored to default staining conditions using the manufacturer's customizations (if available).

f. Backup all Protocols, Reagents and Protocol Customizations files

All protocols, reagents and customizations are stored on the system PC. It is good practice to create a backup copy of these essential files and save onto an external hard drive, USB flash drive, PC, cloud storage, etc.

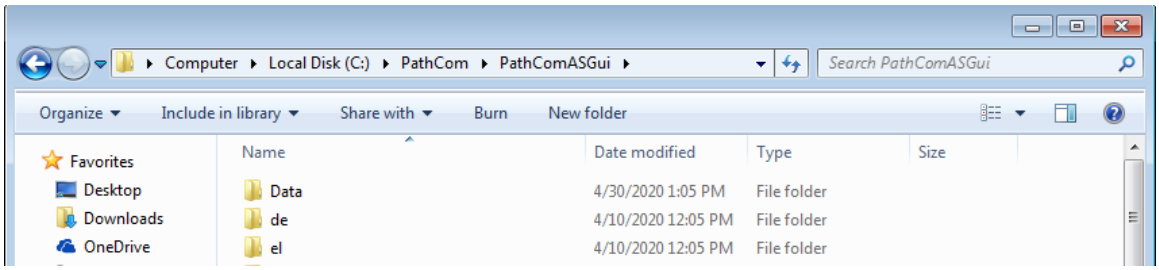


Figure 10

Go to **C:\PathCom\PathComASGui\Data** and create a copy of the following files:

- Protocols.xml
- Reagents.xml
- CstmzProtocols.xml
- CstmzReagents.xml
- DefaultProtocols.xml
- DefaultReagents.xml
- NegativeControl.xml
- templatecfg.dat
- Probe1ExtraWashCycles.xml
- Probe2ExtraWashCycles.xml
- ProtocolEditingOptions.xml
- (If applicable) CustomGroupsAndPanels.xml
- (If applicable) CustomGroupOptions.xml

2.2 Protocol Editor Configuration

The user may customize the display configuration of the main Protocol Editor.

Configuring the Protocol Editor

To configure the Protocol Editor, open System Utilities>Editors>**Protocol Editor Config**.

a. Adjust the left-right display order of the Protocol Type tabs using the up/down arrows.
   - *Note: The display order can be changed to prioritize frequently used Protocol Types.*

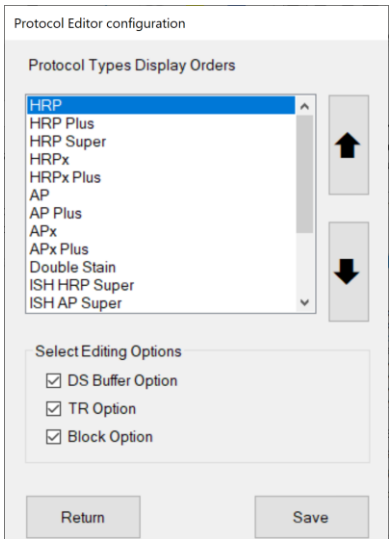


Figure 11

b. Select the Protocol Type from the list and select one or more editing options to display in the tab:
   - DS Buffer Option
   - TR Option
   - Block Option
   - *Note: The Control Reagent option is always displayed and is set by the protocol template.*

Managing Protocol Types

To create a new Protocol Type, open System Utilities>Editors>**Protocol Editor**.

a. Select the Template tab. *Refer to Creating a New Protocol Template for more information.*
b. (Option 1) Click "**Add**" to create a new template and enter a new Protocol Type.
c. (Option 2) Click "**Edit**" to modify an existing template and reassign the template to a new Protocol Type.
   - *Note: All associated protocols will be automatically assigned to the new Protocol Type.*
d. Exit and re-open the Protocol Editor to display the new Protocol Type tab.

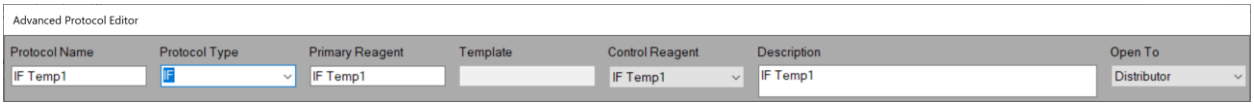


Figure 12

To delete an existing Protocol Type, open System Utilities>Editors>**Protocol Editor**.

a. Select the Template tab.
b. (Option 1) Delete all protocol templates associated with that Protocol Type.
   - *Note: The associated protocols must be deleted before the template may be deleted.*
c. (Option 2) Reassign all protocol templates associated with that Protocol Type to a new Protocol Type.
   - *Note: The associated protocols will automatically transfer to the new Protocol Type's tab.*

Security

Certain features of the Protocol Editor are restricted based on the user's security access level.

Feature	Distributor	Supervisor
Create a standard protocol	✓	✓
Edit a standard protocol	✓	✓
Delete a standard protocol	Except protocols Open To "Closed"	Only protocols Open To "Customer"
Create a special protocol	✓	×
Edit a special protocol	Except protocols Open To "Closed"	Only protocols Open To "Customer" or Open To "Distributor" with Edit Mask enabled
Delete a special protocol	Except protocols Open To "Closed"	×
Create a template	✓	×
Edit a template	Except protocols Open To "Closed"	×
Delete a template	Except protocols Open To "Closed"	×
Create a protocol type	✓	×
Configure Protocol Editor	✓	✓

Section 3: Creating Templates and Special Protocols

The Advanced Protocol Editor enables trained applications specialists to modify/create protocol templates and special protocols.

Click  and login to the application using a Distributor-level account.
Default User ID: Distributor
Default Password: Distributor

Please consult Technical Support before attempting to make any major changes to protocols or reagents. *The default protocols and templates have been carefully optimized to work with the instrument and its proprietary reagents. Certain changes may have unanticipated effects on staining area/quality and runtime.*

3.1 Protocol Templates

Overview of Protocol Templates

A protocol template is a protocol used as a base to generate standard protocols.
Each standard protocol is generated by making a copy of the protocol template, replacing the primary reagent, and applying protocol customizations.

All protocol templates are grouped under the Protocol Type, Template. Additionally, each protocol template is assigned to a Protocol Type to which it can be used to generate standard protocols. The user may apply additional customization to a subset of protocols within a Protocol Type by creating a custom protocol template.

Creating a New Protocol Template

(Option 1) To create a template from an existing protocol or protocol template, open System Utilities>Editors>**Protocol Editor**.

a. Select the Template tab, and right-click>copy and paste an existing template.
b. (Alternatively) Select any tab in the Protocol Editor, including Special, to right-click>copy and paste an existing protocol into the Template tab.

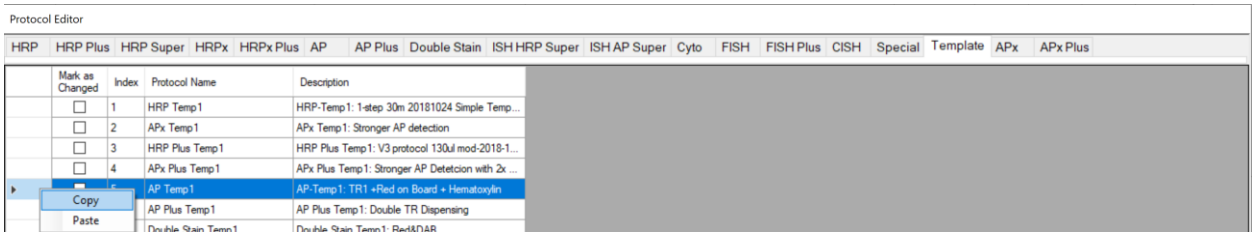


Figure 13

c. The system will generate a default template name. Edit the name as needed, then click "**OK**".
   - **Note: The protocol template name must be unique and must contain the word, " Temp"**
   - *Tip: The word, "Temp", is preceded by a space.*

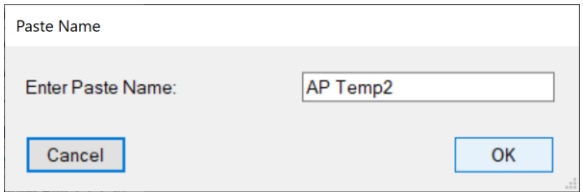


Figure 14

d. Select the template and click "**Edit**" to open the Advanced Protocol Editor.

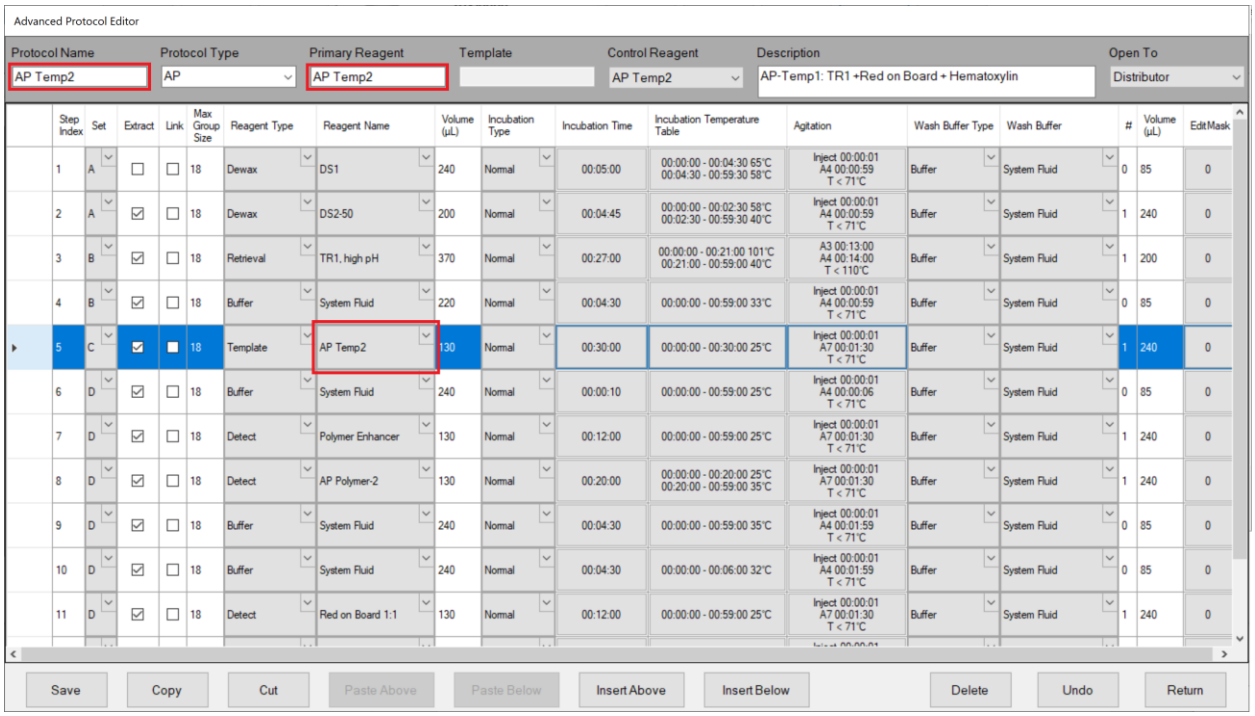


Figure 15

e. Edit the template properties, as needed.

- **Protocol Name**. Edit the template name, as needed.
- **Protocol Type**. Assign the Protocol Type or create a new Protocol Type; the template can only be assigned to the protocols listed under the same Protocol Type tab of the Protocol Editor.
- **Primary Reagent**. The Primary Reagent is automatically set to be the same as the template name.

  Note: The template's primary reagent must be set to the template name. *The template name is used as a placeholder in the body of the protocol template, indicating the position to insert the protocol's primary reagent when the template is used to generate the protocol.*

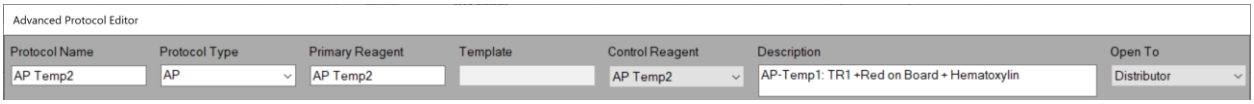


Figure 16

- **Control Reagent**. Assign the Control Reagent; the reagent will appear as a customization option in the Protocol Editor. The user may adjust the incubation time and temperature of the control reagent.
  Note: For IHC applications, set the Control Reagent to the be same as the Primary Reagent. For ISH applications, set the Control Reagent to be the enzyme. For other applications, select any reagent listed in the protocol, excluding: System Fluid, DS option Reagents, TR option reagents and Block option reagents.
- **Description**. Edit the template description. Enter the template name into the description, so that the template can be identified in the Protocol Editor.
- **Open To**. Set the security access level to Distributor to protect the template from deletion by the customer/Supervisor-level user accounts.

f. Edit the protocol Steps, as needed. *Refer to Editing Protocol Steps for more details.*

- **Note: Select Reagent Type, Template, and select the template name to indicate the primary reagent step for the template.**

Figure 17

g. Click "**Save**" and "**Return**".

(Option 2) To create a new template, open System Utilities>Editors>**Protocol Editor**.

a. Select the Template tab and click "**Add**".

b. Enter the new protocol name and click "**Add**" to open the Advanced Protocol Editor.

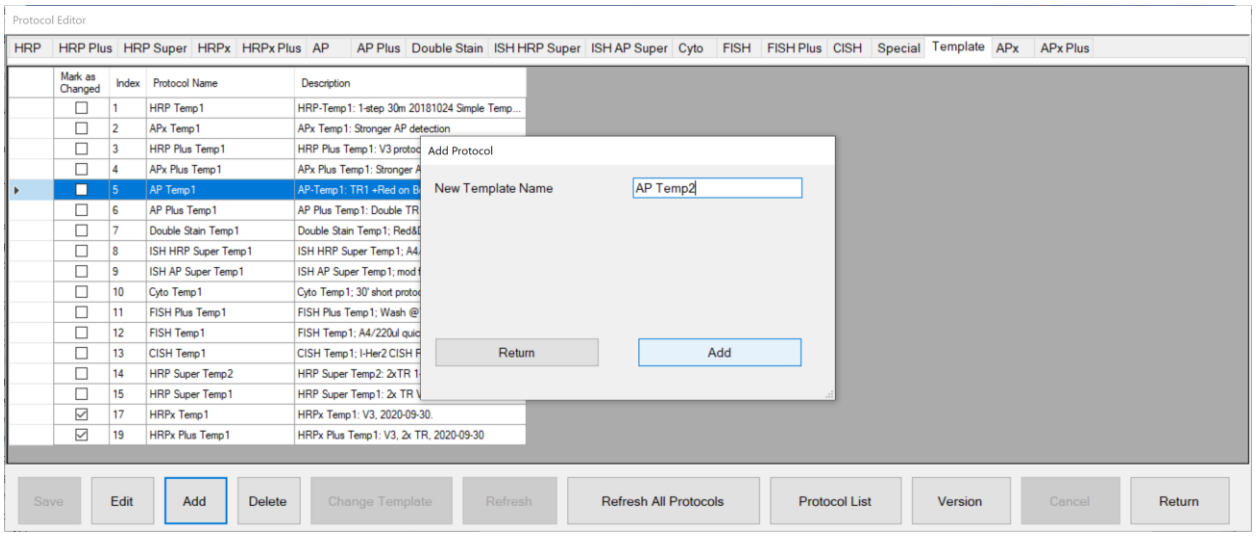


Figure 18

c. The system will automatically generate a basic one-step protocol template with the Primary Reagent automatically added.

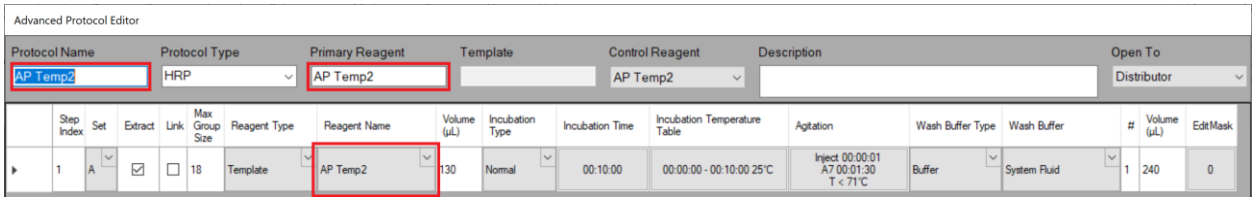


Figure 19

d. Edit the template properties, as needed.

e. Edit the protocol Steps, as needed.

f. Click "**Save**" and "**Return**".

Deleting a Protocol Template

To delete an existing protocol template, open System Utilities>Editors>**Protocol Editor**.

a. Select the template in the Template tab and click "**Delete**".

b. *Note: The system will not permit the deletion of a template that is currently assigned to any standard protocols. Delete the associated protocols or assigned a different protocol template before proceeding.*

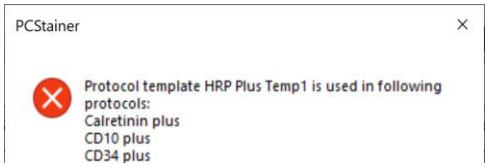


Figure 20

Modifying a Protocol Template

To edit an existing protocol template, open System Utilities>Editors>**Protocol Editor**.

a. Select the Template tab.

b. Select an existing template from the list and click "**Edit**" to open the Advanced Protocol Editor.

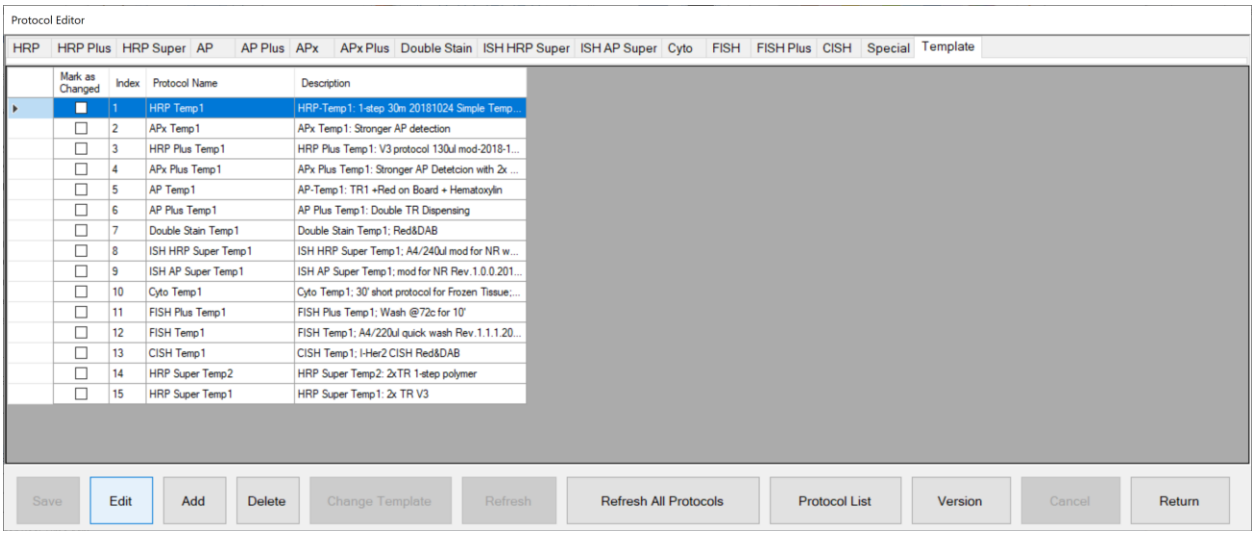


Figure 21

h. Edit the template properties, as needed. *Refer to Creating a New Protocol Template for more details.*

i. Edit the protocol Steps, as needed. *Refer to Editing Protocol Steps for more details.*

c. Click "**Save**" and "**Return**".

d. Select the Refresh Protocol Option: "Refresh with save customization" or "Refresh with manufacturer default".

e. The system will automatically regenerate all related protocols to apply the changes.

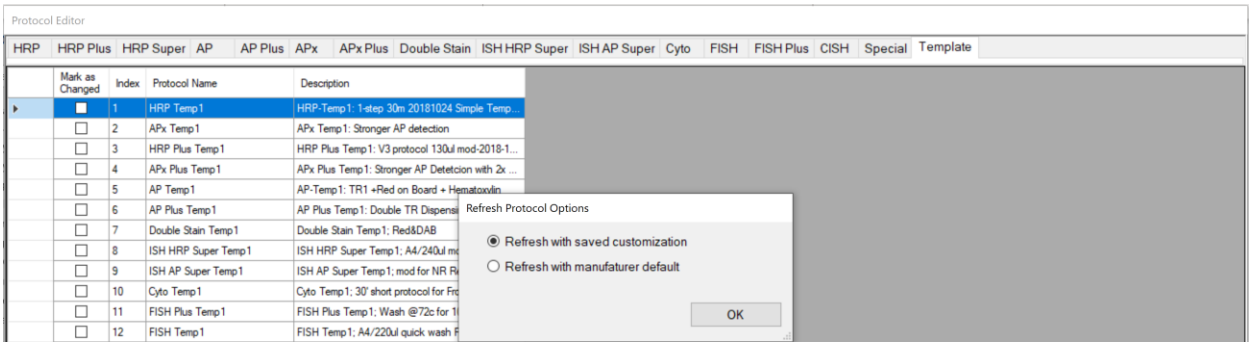


Figure 22

3.2 Special Protocols

Overview of Special Protocols

A special protocol is a fully custom stand-alone protocol.

In contrast to standard protocols, a special protocol is not generated from a protocol template and may be customized on every step of the staining protocol. The user may create special protocols to test difficult antibodies or probes, Special Stains, and other novel staining applications that require customization beyond the scope of the main Protocol Editor.

All special protocols are grouped under the Protocol Type, Special.

Creating a New Special Protocol

(Option 1) To create a special protocol from an existing protocol or protocol template, open System Utilities>Editors>**Protocol Editor**.

a. Select any Protocol Type tab, and right-click>copy and paste an existing protocol or template into the Special tab.

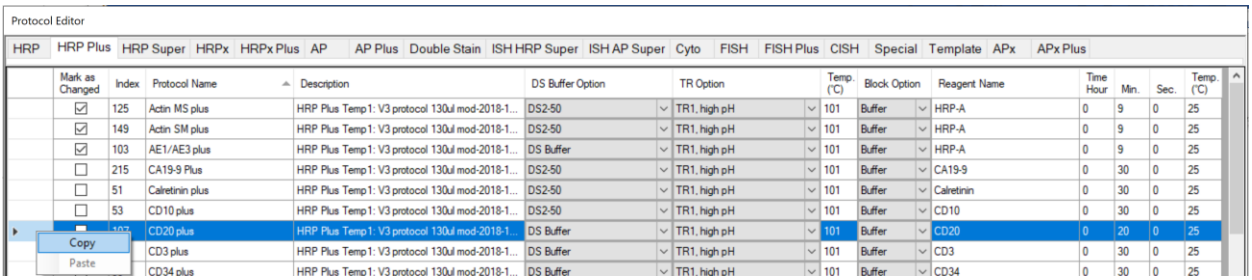


Figure 23

b. The system will generate a default template name. Edit the name as needed, then click "**OK**".
   - *Note 1: The protocol must have a unique protocol name.*
   - *Note 2: To avoid conflicts with positive or negative controls in the programming software, please refrain from using the symbols "+" and "-" at the end of the protocol name.*

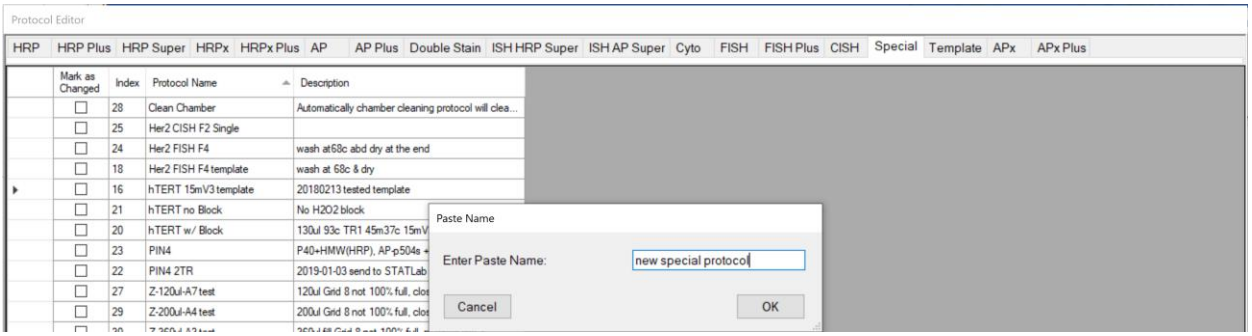


Figure 24

c. Select the protocol and click "**Edit**" to open the Advanced Protocol Editor.
d. Edit the protocol properties, as needed.

1) **Protocol Name**. Edit the protocol name, as needed.
2) **Protocol Type**. The Protocol Type is automatically set to Special.
3) **Primary Reagent**. The Primary Reagent is automatically set to "Unknown" because special protocols are not generated from a template.

Figure 25

4) **Control Reagent**. The Control Reagent is not set, because special protocols are not customizable in the main Protocol Editor.
5) **Description**. Edit the protocol description, so that the protocol can be identified in the Protocol Editor.
   6) **Open To**. Set the security access level to Distributor to protect the protocol from deletion by the customer/Supervisor-level user accounts.

e. Edit the protocol Steps, as needed. *Refer to Editing Protocol Steps for more details.*
f. Click "**Save**" and "**Return**".

(Option 2) To create a new special protocol, open System Utilities>Editors>**Protocol Editor**.

a. Select the Special tab and click "**Add**".
b. Enter the new protocol name and click "**Add**" to open the Advanced Protocol Editor.

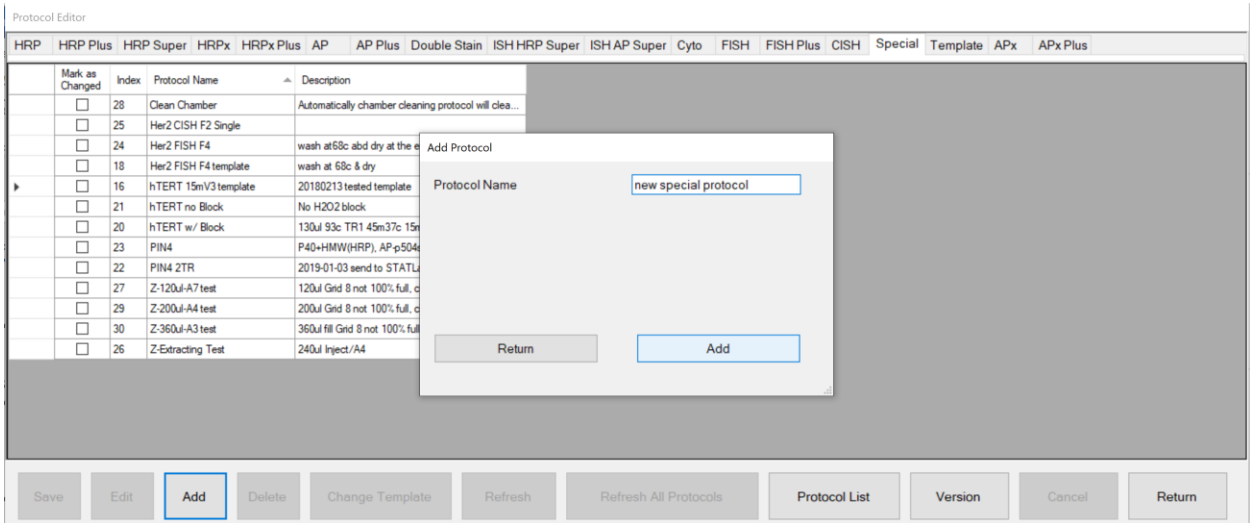


Figure 26

c. The system will automatically generate a basic one-step protocol template with the Primary Reagent automatically added.

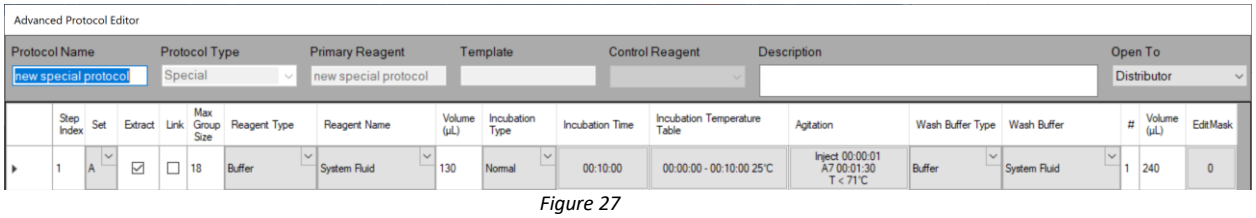


Figure 27

d. Edit the protocol properties, as needed.
e. Edit the protocol Steps, as needed.
f. Click "**Save**" and "**Return**".

Deleting a Special Protocol

To delete a special protocol, open System Utilities>Editors>**Protocol Editor**.

a. Select the protocol in the Special tab and click "**Delete**".

Modifying a Special Protocol

To edit an existing special protocol, open System Utilities>Editors>**Protocol Editor**.

a. Select the Special tab.
b. Select a special protocol from the list and click "**Edit**" to open the Advanced Protocol Editor.

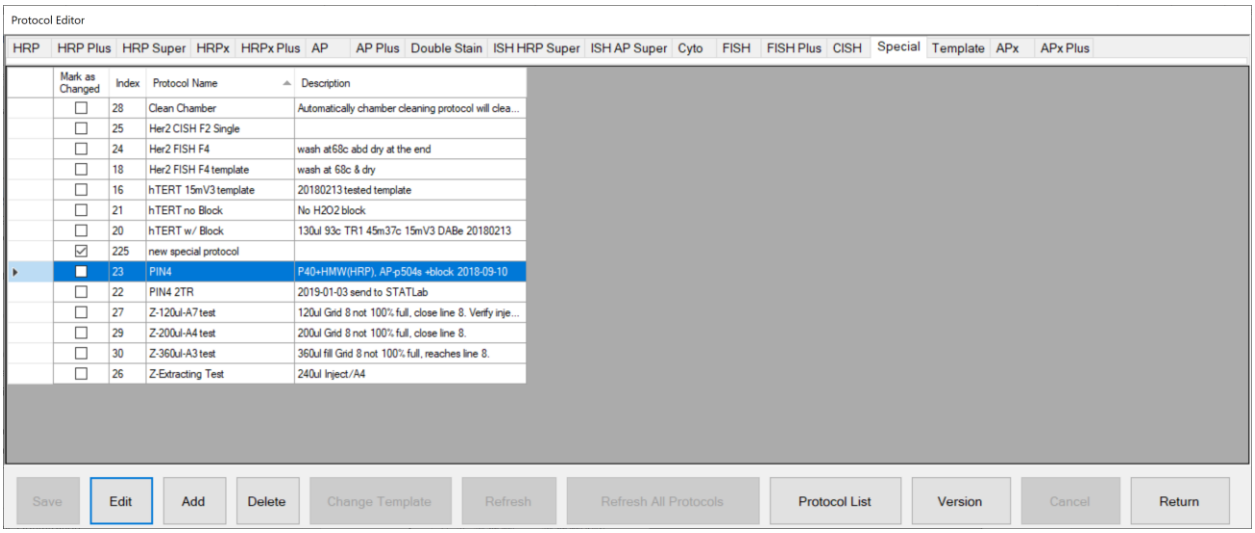


Figure 28

c. Edit the protocol properties, as needed. *Refer to Creating a New Special Protocol for more details.*
d. Edit the protocol Steps, as needed. *Refer to Editing Protocol Steps for more details.*
e. Click "**Save**" and "**Return**".

Editing Protocol Steps

Each Step of a special protocol or template may be customized in the Advanced Protocol Editor.
Note: Standard protocols are generated from a template and cannot be edited in the Advanced Protocol Editor. Change the protocol template or create a special protocol if further customization is required.

To define a protocol Step, set the protocol parameters. *Refer to Protocol Parameters for more details.*

a. Assign the **Set** to identify and pool common steps between protocols, ie: A, B, C, D.
b. Select the **Extract** option to extract the waste before injecting the new reagent.
c. Select the **Link** option to link the current Step to the previous Step.
d. Set the **Max Group Size** to set the maximum number of slides that may be pooled and run together as a group.
e. Select the **Reagent Type** and **Reagent Name** and set the **Volume(uL)**.
f. Set the **Incubation Type** to control the incubation accuracy: Normal, Fine, AirDry, Accurate (in development).
g. Set the **Incubation Time** (total controlled incubation time). *Note: Set the time to 00:00:00 for No Incubation.*
h. Setup the **Incubation Temperature Table** to control the heating during the incubation period.
i. Set the **Agitation** to control the lid movement for the incubation period.
j. Select the **Wash Buffer Type** and **Wash Buffer** and set the **#** and **Volume(ul)** of Quick Washes to execute at the end of the Step. *Note: Set the # to 0 for No Wash.*

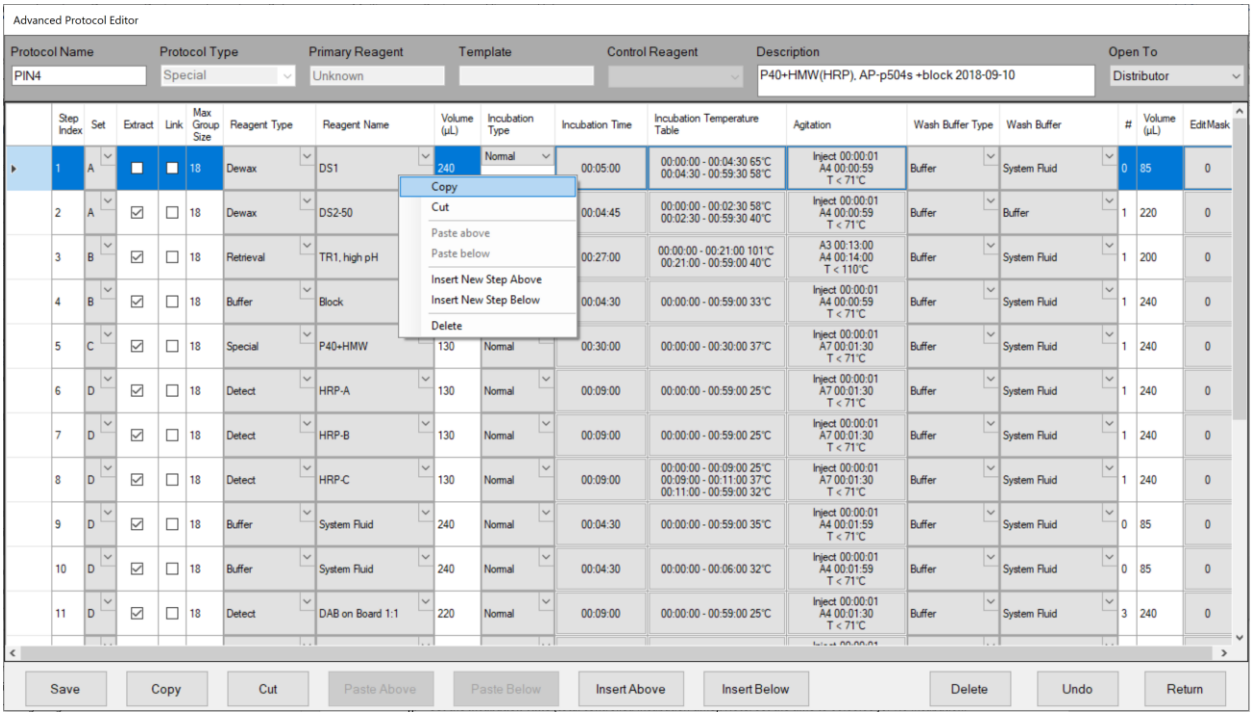


Figure 29

To add, remove and move protocol Steps, right-click or use the editor buttons.

a. Click "**Insert Above**" or "**Insert Below**" to add a step.
b. Select one or more steps and click "**Delete**" to remove a selection of protocol steps.
c. Select one or more steps and click "**Copy**" to copy a selection of protocol steps.
d. Select one or more steps and click "**Cut**" to cut a selection of protocol steps.
e. Click "**Paste Above**" or "**Paste Below**" to paste a selection of steps.

To undo all changes since the last save, click "**Undo**".
To save changes to the protocol, click "**Save**".

3.3 Protocol Parameters

A protocol is a unique sequence of Steps (reagent incubation steps and wash steps) that the system is programmed to execute during the staining process. Each protocol Step is defined by several user-configurable parameters.

Figure 30

Set

Assign Set A, B, C, D,..etc, for each Step to identify and group common Steps amongst individual staining protocols. Up to 36 different protocols may be run simultaneously during one staining run; therefore, it is important that common Steps may be pooled and executed together efficiently. This feature is primarily used to optimize runtime scheduling, and to ensure that certain sections of the protocol(s) are executed for all slides before progressing to the next section.

Note: It is recommended to set the same standard RunSet pattern ie: AABBC(D variable) across all protocols and protocol templates to ensure that they can be run together efficiently in the same staining run.

Extract

Select the option to extract/not extract the waste from the previous Step, before executing the current Step.

1) The option to extract waste is selected by default.
2) The option not to extract waste may be selected for on-slide mixing of reagents or other special cases.

Link

Select the option to link the current Step to the previous Step.

Linked Steps are prioritized by the runtime schedule and will be executed consecutively. The Linked Step will initiate immediately upon completion of the previous Step. This will eliminate “wait time” and interruptions caused by the other processes that are running simultaneously in the same staining run.

Note: Selecting this option may negatively affect overall runtime as it reduces scheduling flexibility.

1) The option to link steps is deselected by default.
2) The option to link steps may be selected to enforce the set incubation time for time-sensitive protocol steps.

Max Group Size

Set the maximum group size to adjust the incubation accuracy of the current Step.

The run scheduler will pool common steps between protocols and execute those slides together. Set the group size smaller to reduce the reagent dispensation time, and thereby the “effective” incubation time, to improve the incubation accuracy of time-sensitive reagents.

Note1: The other Steps in the same Set will also be set to the same group size.

Note2: Setting a smaller group size will negatively affect overall runtime by increasing the scheduling complexity.

1) The maximum group size is set to default 18 for a standard 36 slides instrument.
2) The maximum group size is set to default 12 for a 12/24 slides instrument.
3) The maximum group size is set to default 9 for a Special Stains instrument, incubation time variation <2min.

Reagent Type and Reagent Name

Select the reagent applied on the current Step. Reagents are categorized by Reagent Type

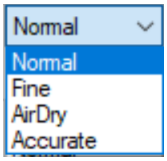
1) Select a reagent to execute a Reagent Incubation Step.
2) Select System Fluid or other wash solution to execute an Incubated Wash Step.
   Note: This option may be used to improve wash efficacy by adding time, heat and agitation.
   a. *(Optional) Select the Link option (to execute immediately after the previous Step, like a Quick Wash)*
   b. *Select the Reagent Type and Reagent Name of the wash solution. Set the Volume(uL): 240uL.*
   c. *Set the Incubation Type: Normal. Set the Incubation Time and Incubation Temperature Table.*
   d. *Set the Agitation Parameters: Inject/A4 (1s/1min59s).*

Volume(uL)

Set the volume of reagent or wash solution applied on the current Step.
A standard volume of 130uL (in 100uL chambers) and 200uL (in 200uL chambers) is necessary to achieve acceptable staining area and uniform coverage for most reagents. The user may set a slightly larger volume for washes (220-240uL) and heated incubations by selecting the appropriate agitation angle. The user may set a slightly smaller volume following a Step that may be affected by insufficient extraction, ie: TR.
Note1: Consider the agitation parameters, incubation time and temperature, and rate of evaporation when determining the appropriate volume of reagent to use. Note that the actual volume of reagent drawn and dispensed is affected by the reagent's viscosity properties. Reagent handling is optimized for each viscosity level to maintain consistency between slides.
Note2: The chambers are theoretically designed to hold a minimum volume of 85uL (small size, red) and 150uL (large size, blue); however, this is generally not put into practice.

Incubation Type

Select the type of incubation to be executed on the current Step.



1) **Normal** incubation. Perform a timed incubation with controlled heat and agitation. The incubation accuracy will be affected by several factors: 1) group size and reagent dispensation time, 2) Quick Wash option, 3) Link option in the next Step, and 4) scheduling of the next Step.
2) **Fine** incubation. Precisely control the incubation time <1min, minimum 10s. The incubation will be executed one-by-one for each slide in the group to ensure the accuracy of the incubation. System fluid is dispensed at the end of each process.
   Note1: This option is designed for very short incubation times. The system will remain idle to ensure that the robot is readily available. No other protocol Steps or processes may be carried out during this time. Consider reformulating reagent solutions if the incubation time exceeds 30s-1min, as this may greatly increase the overall runtime.
   Note2: This option may generate some non-uniformity for particularly time-sensitive reagents <1min, especially between the upper and lower half of the slide due to the difference in reagent coverage during reagent injection.
   The following will be carried out for each slide in the group:
   a. The robot will aspirate one test from the reagent vial and dispense it to the slide.
   b. The reagent will incubate for the exact time set by the Total Incubation Time.
      The lid will remain at the lower lid position with no agitation.
      The incubation temperature of the previous Step will carry over to the current Step.
c. The robot will immediately extract the reagent waste from the slide upon completion of incubation, and promptly wash the slide with System Fluid.
3) **AirDry** incubation. Perform a Fine incubation with drying. The incubation will be executed one-by-one for each slide in the group to ensure the accuracy of the "effective" incubation time (within seconds of the set incubation time). System Fluid is NOT dispensed at the end of each process.
   Note: This option is designed for reagents that are particularly soluble in water ie: aqueous Light Green.
4) **Accurate** incubation. Precisely control incubation time >1min.
   Note: This feature is currently in development and is not available at this time.

Incubation Time

Set the "total" incubation time, the period between the start and end of the current Step.

Note1: The antibody incubation time for IHC Protocols and Pepsin incubation time for ISH Protocols must be between 5-120 minutes.

Note2: Consider incubation accuracy when setting short incubation times. Consider the impact on staining area when setting long incubation times, especially for heated processes.

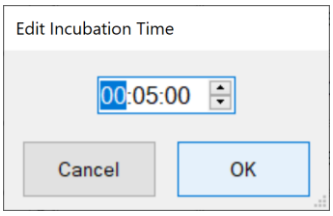


Figure 31

a. The incubation time starts after reagent has been dispensed to the last slide in a pooled group.
b. During incubation, the reagent will incubate on the slide while the system controls heating and agitation. The user must allot sufficient time for active heat up and passive cool down to the temperature set point.
c. The incubation time ends after the total incubation time has elapsed. The system will stop agitation and may 1) proceed immediately to extract waste and perform the Quick Wash*, or 2) maintain a set temperature and wait until the run schedule reaches the next Step of the protocol.
d. For time-sensitive reagents, it is important to consider the total time exposed to the reagent will be longer than the set incubation time. The "effective" incubation time" is affected by several factors:
   The reagent dispensation time.
   - Reagent handling properties; certain reagents may take longer (ie: on-rack mixing reagent) or shorter (ie: no extraction) to dispense to the slides.
   - Pooled group size; the robot will take a longer time (up to 4min) to dispense to a larger group of slides (up to 18) compared to a smaller group of slides, or individual slide.

   The scheduled "wait time" between the end of the current incubation and the beginning of the next Step when the reagent waste is extracted.
   - Quick Wash*; selecting one or more Quick Washes to execute at the end of the Step will ensure that the reagent waste is removed promptly at the end of incubation and replaced with a buffer or other neutral solution that will not affect the staining.
   - Linked Step; linking the next Step in the protocol will ensure that the reagent waste is removed promptly at the end of incubation.
   - Run scheduling; if neither a Quick Wash nor a Linked Step is selected, the reagent will remain on the slide until the next Step is executed, which may vary from a few minutes to a few hours later, based on the system's calculation for the most efficient schedule.

   Note: Increasing the incubation accuracy will reduce runtime flexibility and have a negative effect on overall runtime. Adjust the reagent concentration (or temperature) to minimize the impact of the "effective incubation time". Certain reagents may be adjusted to reach saturation levels. Certain reagents may be diluted to increase the incubation time sufficiently, so that the effect of a few minutes of additional time is negligible.

Relationship of Incubation Time, “Effective” Incubation Time, Heat Incubation Time

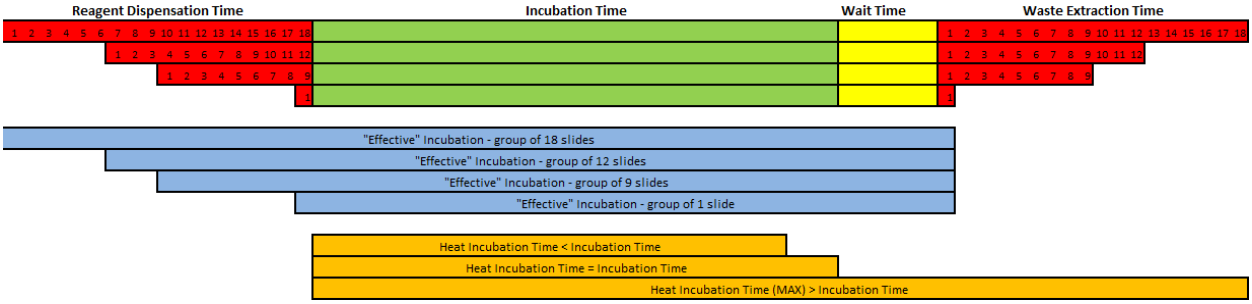


Figure 32

Incubation Temperature Table

Setup one or more heating periods to precisely control the temperature (°C) at specific time points during the incubation.

Note1: The antibody heating time for standard IHC Protocols/pepsin heating time for ISH Protocols is automatically set to equal the total incubation time.

Note2: Maximum temperature set point is 110 ℃.

Note3: Minimum temperature set point is the ambient room temperature (no active cooling capability).

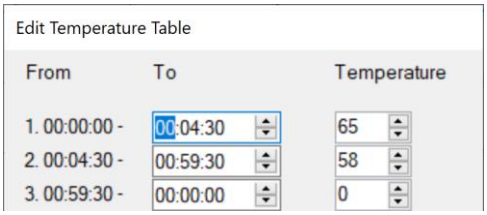


Figure 33

a. Click the Temperature Table cell to display the editing options.

b. Setup the heating period(s) From/To on the Temperature Table.
   - The first heating period starts after reagent has been dispensed to all slides in a pooled group.
   - The next heating period will start immediately after the previous heating period finishes.
   - The user may set up to 10 heating periods within the incubation.
   - *Note1: The heating period must include the time needed to heat up to the set temperature. The system may take a few minutes to reach a high temperature from RT through active heating.*
   - *Note2: The heating period must include the time needed to cool down to the set temperature. The system may take a significant time to reach RT through passive cooling.*

c. The heating period may end at 1) a point during the incubation, 2) the end of the incubation, 3) at a point between the end of the incubation and the start of the next Step of the protocol.
   - To incorporate a cooling period, start the last heating period within the incubation and set a lower temperature.
   - To incorporate pre-heating period before the next step, start the last heating period within the incubation and set a higher temperature.
   - To incorporate a heated Quick Wash after the incubation, start the last heating period after the incubation and set a higher temperature.
   - To incorporate a maintaining temperature period, start the last heating period within or after the incubation and set the temperature.

Heating/Cooling Curve of a Standard Heater (via External Temperature Sensor)

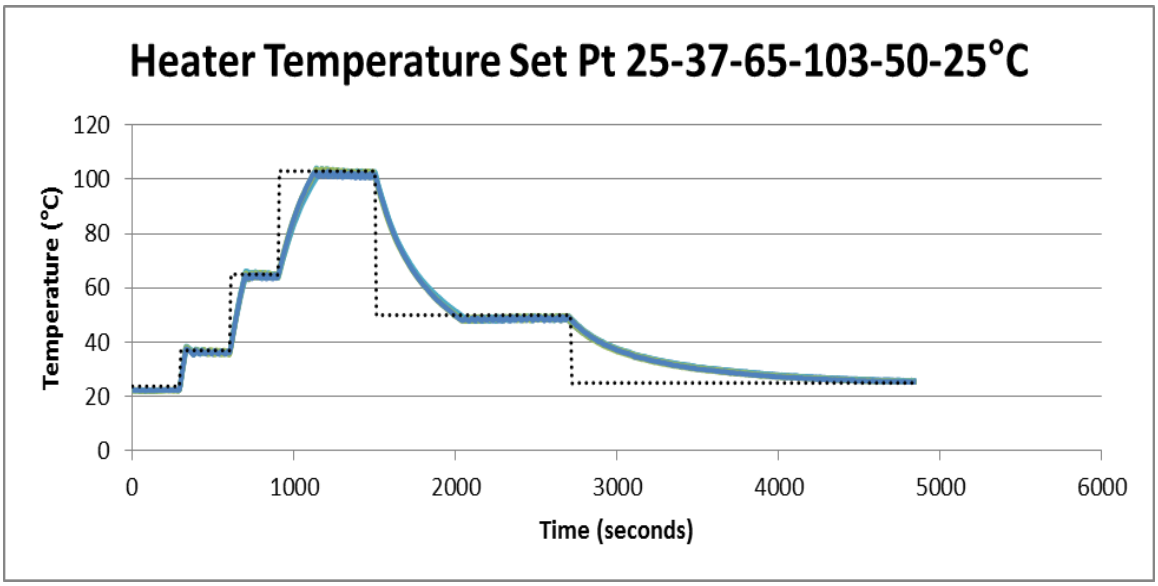


Figure 34

Agitation Parameters

Setup the agitation parameters. The module lids will alternate between two angles, **Lid Up Position** and **Lid Down Position**, at a certain frequency, determined by the **Lid Up Time** and **Lid Down Time**. This mechanism helps to accelerate the rate of reaction, improve uniformity, and disperse air bubbles. Additionally, set the **Threshold** temperature to stop agitation above a certain temperature set point.

Note: Consider the overall incubation time and temperature when determining the optimum agitation frequency and positions for each Step. More frequent agitation and higher agitation positions may improve the effectiveness of mixing and washing but will also increase the rate of evaporation and increase the potential for reagent leaking from the slide. Overly aggressive agitation may even wash away the signal from the tissue in certain applications. Lower agitation positions may decrease the effect of evaporation, but may intensify the negative effects of air bubbles, reagent leaking and staining unevenness. Higher agitation positions may be used to accommodate larger volumes of liquid to limit the effect of evaporation but must be paired with lower agitation frequencies to limit reagent leaking. The user must balance the tradeoffs between all factors to achieve an optimal agitation cycle.

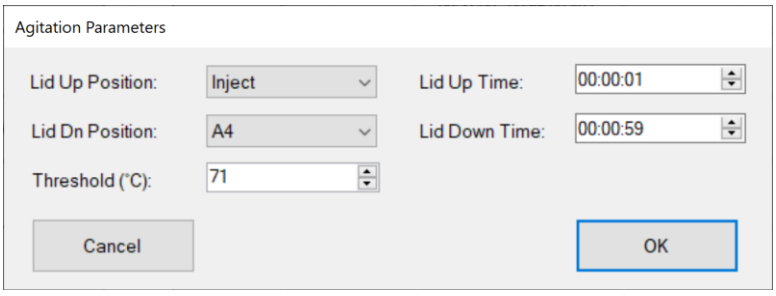


Figure 35

a. Click the Agitation cell to display the editing options.
b. Set the Agitation Cycle to control the lid movement during the incubation.
   Note1: Exceeding the agitation parameters as defined below may result in software crashes/failures.
   Note2: If Total Incubation Time = 0s, 0 agitation cycles will be executed.
   Note3: Lid Up Time + Lid Down Time must be <= Total Incubation Time. If Lid Up Time + Lid Down Time > Total Incubation Time, 0 agitation cycle will be executed.
   Note4: The lid will remain at the lower lid position at the end of the incubation.

- **Lid Up Time** (minimum 1s, maximum 54 minutes 36 seconds)
- **Lid Down Time** (minimum 1s, maximum 54 minutes 36 seconds).
- **Lid Up Position** (*cannot exceed 700 steps)*
- **Lid Down Position** (*cannot exceed 100 steps). Lid Down Position must be < Lid Up Position.*

c. Set the Threshold(°C) Temperature to stop/resume lid movement after reaching a temperature set point during the incubation. This option may be used to reduce evaporation when incubating at high temperatures or for long time periods, ie: FISH probe hybridization; however, it may affect uniformity.
   - Agitation will stop and the lid will go to the **Down** position to seal the slide when the temperature exceeds the threshold temperature.
   - Agitation will resume when the temperature falls below the threshold temperature.

In general, the relative angles of the agitation positions will range as follows:
Home>>>A1>>Extract>Inject>A2>A3>A4>A5>A6>A7>Down

1) Home; maximum lid angle/fully open, triggers the home sensor, Range 500-700 steps
2) A1; upper lid angle for reagent mixing step or drying step, Extract +100 steps
3) Extract; upper lid angle for reagent dispensation/waste extraction
4) Inject; upper lid angle (most frequently used), Extract -20 steps
5) A2; A3+4 steps
6) A3; upper lid angle for 360-400uL target retrieval step
7) A4; lower lid angle for 200-240uL washes and reagents
8) A5; *not implemented at this time*
9) A6; *not implemented at this time*
10) A7; lower lid angle for 100-130uL reagents (100uL chamber) OR 200uL reagents (200uL chamber)
11) Down; minimum lid angle/fully closed and sealed, 0 steps

Liquid coverage at indicated calibration volumes 360, 220 and 120uL for a standard 130uL chamber:

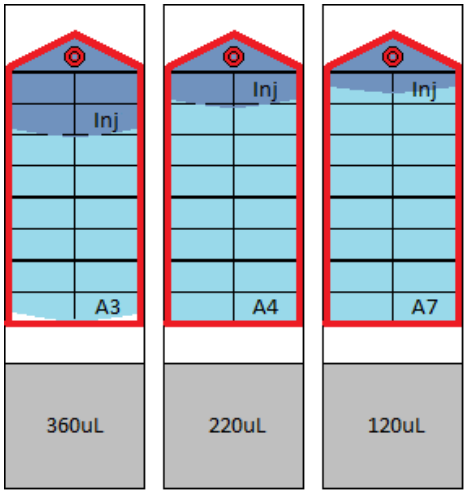


Figure 36

Note: The actual values of agitation angles will vary based on the calibration of each staining module.

Please consult Technical Support for more details before making changes to the agitation positions.

Wash Buffer Type and Wash Buffer

Select the reagent applied for the Quick Wash at the end of the current Step.
The default wash solution is System Fluid (in-line buffer).
Note1: The user may select a wash solution from any Reagent Type.

and Volume(uL)

Set the number of iterations and volume of wash solution to apply for the Quick Wash.
Immediately upon completing the incubation of the current Step, the robot will extract the reagent waste and apply the set volume of wash solution. The lid will quickly agitate two times between upper lid angle, Inject, and lower lid angle, A4*, and then remain at the lower lid position, A4*. The process will repeat for the number of iterations specified. The slide will sit in the wash solution until it is extracted in the next protocol Step.
Note1: The standard wash is set to volume 240uL, for upper lid position Inject, lower lid position A4.*
Note2: The setting for the Quick Wash's lower lid position, A4, is configured by the Global Parameters.*
Note3: Setup a Quick Wash for time-sensitive reagents, or to add washes without adding additional Steps which would affect the RunSet pattern. Otherwise, setup an Incubated Wash to improve runtime flexibility, or to implement a more effective wash.

Edit Mask

Setup the available editing options for each Step in the staining protocol.
This is a security feature for the Advanced Protocol Editor.
Note: Only applicable to Special protocols designated Open To "Distributor".

1) Edit Mask value=0 indicates that the Step cannot be edited by Supervisor-level users.
2) Edit Mask value>0 indicates that certain parameters in the Step can be edited by Supervisor-level users.

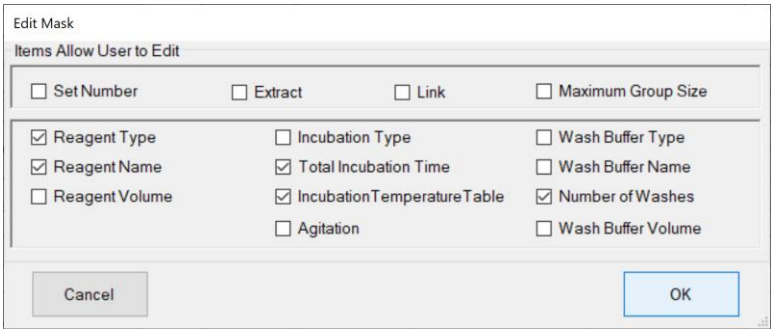


Figure 37

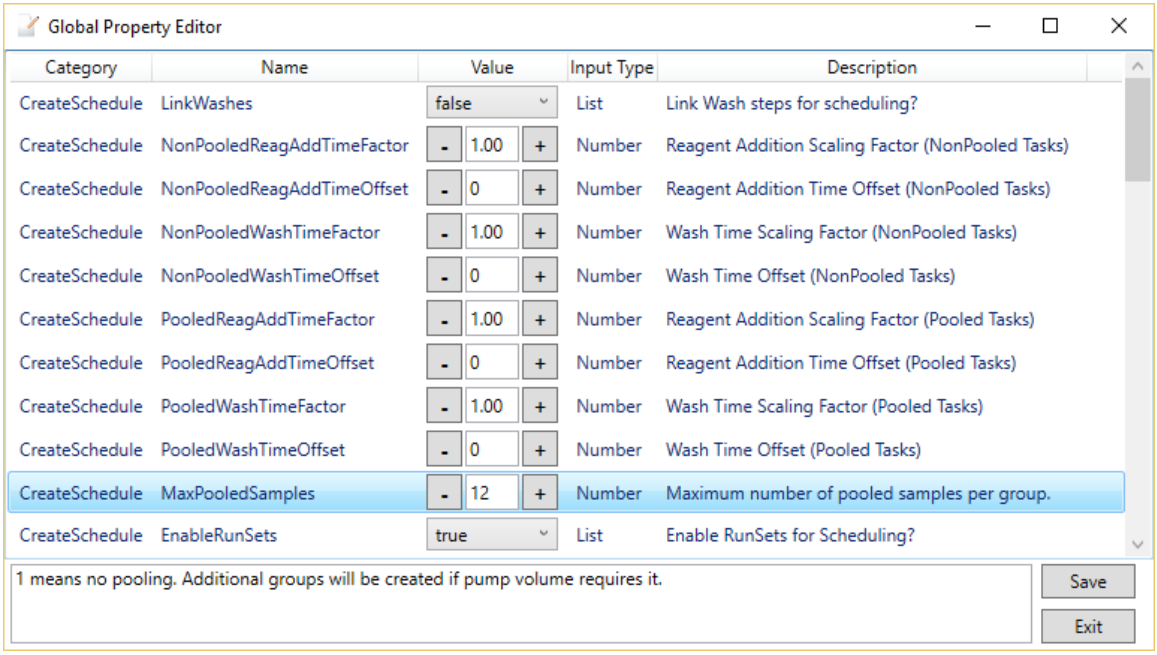
a. Click the Edit Mask cell to display the editing options.
b. Select the editing options to make available to the Supervisor.
c. All other options are deselected/restricted by default.

3.4 Global Parameters

The global parameters control the liquid handling settings for the entire system.
These settings are pre-configured for each system and should not be modified by the user.

Do not modify the global parameters without consulting Technical Support.

To view the global parameters, open C:\PathCom\PathComAutostainer\GPEditor.exe.



MaxPooledSamples (9 or 12 or 18, default). The maximum number of slides per group.

SlideModuleVolume (270, default). The extraction volume(uL) for each slide.

Probe1WashCycles* (1, default). The number of Z1 probe washes. The outer surface of the probe must be washed at the wash station after reagent dispensation to avoid cross-contamination between reagent vials.

Probe1RinseCycles (3, default). The number of Z1 probe rinses. The inner surface of the probe and tubing must be rinsed at the waste station after reagent dispensation to avoid waste build-up and contamination of aspirated reagents within the tubing.

Probe2WashCycles* (1, default). The number of Z2 probe washes. The outer surface of the probe must be washed at the wash station after reagent dispensation to avoid cross-contamination between reagent vials (on-board mixing only). The outer surface of the probe must be washed after waste extraction to avoid cross-contamination of modules (waste extraction of antibody only).

Probe2RinseCycles (2, default). The number of Z2 probe rinses. The inner surface of the probe and tubing must be rinsed at the waste station after waste extraction to avoid waste build-up and contamination of aspirated reagents within the tubing.

**Note: The user may configure the number of additional wash cycles for any Reagent or Reagent Type.*

a. Go to C:\PathCom\PathComASGui\Data

b. Right-click and modify the following files with a text editor:

- *Probe1ExtraWashCycles.xml*
- *Probe2ExtraWashCycles.xml*

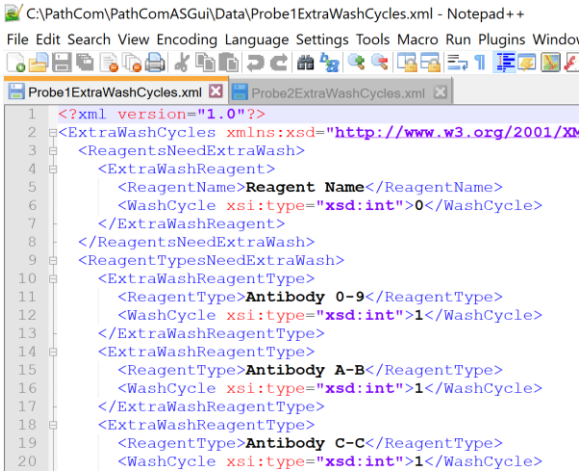


Figure 38

QuickWashLowPos (A4, default for 240uL Quick Wash volume). The lower agitation position for the Quick Wash.
Note: Some systems may be configured to A7, default for 130uL Quick Wash volume.

TubingCleanMethod (1, default). The tubing clean method.

DisplayTime (Remaining, default). The method to display time during the staining run; time remaining or expected finish time.

ProbeWaitingShakingTimeV1-6 (0, default). The amount of time to wait after taking reagent viscosity 1-6(to minimize reagent drip).

VolumeCheck (false, default). Enable/disable the volume check feature during Reagent Check. The system will use the liquid sensor to gauge the volume of reagent remaining in the vial, ie: pre-mixed reagents. This feature only applies to Reagents listed under the Reagent Type, Volume Check.
Note: This feature is disabled by default.

SpecialAndQuickWashUpPos (Inject, default). The upper agitation position for the Quick Wash.

ExpiredReagentAllowed (false, default). Enable/disable the use of expired reagents.

3.5 Runtime Schedule

The runtime schedule is calculated at the start of each staining run and performs the essential function of arranging the order and grouping of Steps for all the running protocols to minimize overall runtime.

Overview

The instrument may execute up to 36 different protocol(s) in tandem within one staining run.
However, the schedule will increase in time and complexity when running a mixed run of protocols with different customizations and/or different Protocol Types, and as the number of slides in the run increases and as the number of Steps in the protocol(s) increases. Therefore, it is important to maximize slide throughput by strategically grouping similar protocols within the same staining run, reserving long protocols for overnight runs.

The system's runtime scheduler will perform a series of simulations on each Set of steps to determine the most efficient sequence of protocol Steps. The scheduler must maximize the available working time of the robotic arm, while minimizing "wait time" between the execution of protocol Steps running in tandem, while controlling the set incubation time of time-sensitive Steps.

a. The system may operate in two running modes:
   - RunSet; the protocol Steps are grouped in Sets that can pool common Steps between protocols
   - Dynamic; the protocol Steps are ungrouped, therefore, each protocol's Steps are run separately
b. The order of Steps as defined by each protocol will be enforced.
c. Identical Steps among different protocols will be pooled, if possible, within the Set.
d. Multiple protocols are executed in tandem.
   - The system can initiate the next Step in the schedule while the previous Step(s) are performing incubation, under the condition that the robotic arm has sufficient time to complete the task.
   - The system may schedule a "wait time", in which the slide(s) will rest in Wash Buffer or other reagent solution (not affected by over-incubation), to allot more time to the robotic arm.
e. Longer protocols will be given priority and will start earlier than shorter protocols.
f. Longer incubation times will be given priority and will start earlier within the Set.

The user may run schedule simulations to calculate the runtime and preview the arrangement of Steps for a set of protocols run together in a staining run.

a. Manually assign protocols and reagents.
b. Start the staining process and wait for the scheduler to complete calculations.
c. Click to expand the Autostainer Server from the taskbar.
d. Select the Gantt tab to view the current schedule.
e. Click the Stop button in the upper right corner to stop the current run.

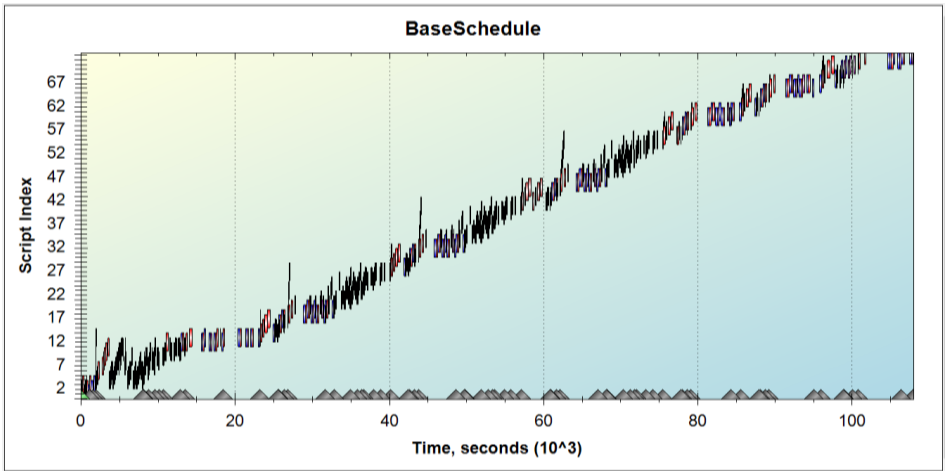


Figure 39

RunSet Mode

This mode of scheduling increases runtime efficiency by pooling the slides to run common protocol Steps together.

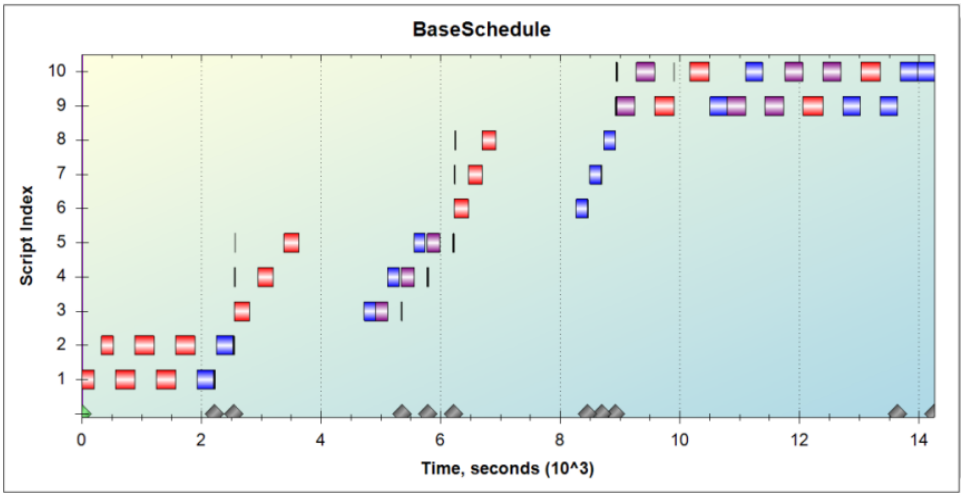


Figure 40

In order for the scheduler to identify common protocol Steps, the Steps of each Protocol are grouped into **Sets**. Only identical Sets may be pooled from different protocols and run together. All protocols must complete all Steps within the current Set, before the system can move on to the next Set. The standard staining protocols are configured to follow a standard pattern of Sets, termed the RunSet, to maximize grouping opportunities.

The RunSet divides the standard IHC staining protocol into 4 Sets:

a. AA, for Dewaxing
b. BB, for Target Retrieval
c. C, Primary Abs
d. DDDDDDDD, for Detection (variable number of steps permitted)

Advanced Protocol Editor

Protocol Name: HRP Plus Temp1 | Protocol Type: HRP Plus | Primary Reagent: HRP Plus Temp1 | Template: | Control Reagent: HRP Plus Temp1 | Description: HRP Plus Temp1: V3 protocol 130ul mod-2018-10-24 | Open To: Distributor

Step Index	Set	Extract	Link	Max Group Size	Reagent Type	Reagent Name	Volume (µL)	Incubation Type	Incubation Time	Incubation Temperature Table	Agitation	Wash Buffer Type	Wash Buffer	#	Volume (µL)	EditMask
1	A	☐	☐	18	Dewax	DS1	240	Normal	00:05:00	00:00:00 - 00:04:30 65°C 00:04:30 - 00:59:30 58°C	Inject 00:00:01 A4 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
2	A	☑	☐	18	Dewax	DS2-50	200	Normal	00:04:45	00:00:00 - 00:02:30 58°C 00:02:30 - 00:59:30 40°C	Inject 00:00:01 A4 00:00:59 T < 71°C	Buffer	Buffer	1	220	0
3	B	☑	☐	18	Retrieval	TR1, high pH	370	Normal	00:27:00	00:00:00 - 00:21:00 101°C 00:21:00 - 00:59:00 40°C	A3 00:13:00 A4 00:14:00 T < 110°C	Buffer	System Fluid	1	200	0
4	B	☑	☐	18	Buffer	System Fluid	220	Normal	00:04:30	00:00:00 - 00:59:00 33°C	Inject 00:00:01 A4 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
5	C	☑	☐	18	Template	HRP Plus Temp1	130	Normal	00:30:00	00:00:00 - 00:30:00 25°C	Inject 00:00:01 A7 00:01:30 T < 71°C	Buffer	System Fluid	1	240	0
6	D	☑	☐	18	Detect	HRP-A	130	Normal	00:09:00	00:00:00 - 00:59:00 25°C	Inject 00:00:01 A7 00:01:30 T < 71°C	Buffer	System Fluid	1	240	0
7	D	☑	☐	18	Detect	HRP-B	130	Normal	00:09:00	00:00:00 - 00:59:00 25°C	Inject 00:00:01 A7 00:01:30 T < 71°C	Buffer	System Fluid	1	240	0
8	D	☑	☐	18	Detect	HRP-C	130	Normal	00:09:00	00:00:00 - 00:09:00 25°C 00:09:00 - 00:11:00 37°C 00:11:00 - 00:59:00 32°C	Inject 00:00:01 A7 00:01:30 T < 71°C	Buffer	System Fluid	1	240	0
9	D	☑	☐	18	Buffer	System Fluid	240	Normal	00:04:30	00:00:00 - 00:59:00 35°C	Inject 00:00:01 A4 00:01:59 T < 71°C	Buffer	System Fluid	0	85	0
10	D	☑	☐	18	Buffer	System Fluid	240	Normal	00:04:30	00:00:00 - 00:06:00 32°C	Inject 00:00:01 A4 00:01:59 T < 71°C	Buffer	System Fluid	0	85	0
11	D	☑	☐	18	Detect	DAB on Board 1:1	220	Normal	00:09:00	00:00:00 - 00:59:00 25°C	Inject 00:00:01 A4 00:01:30 T < 71°C	Buffer	System Fluid	1	240	0

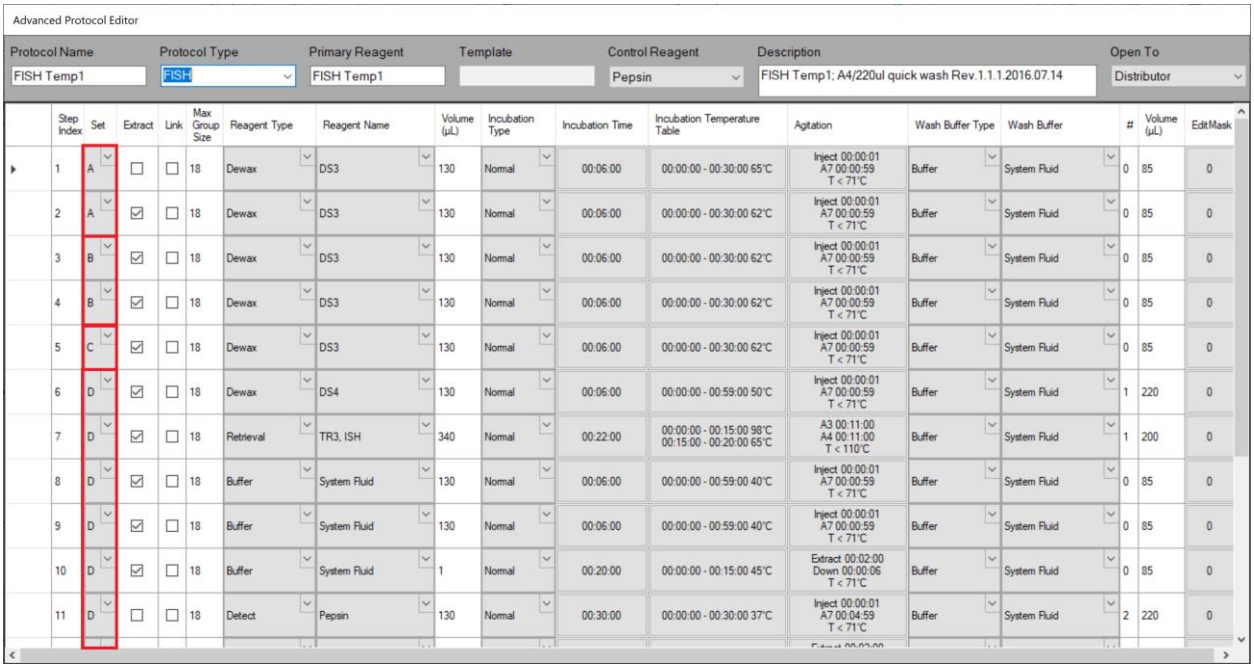
Figure 41

The system is designed to automatically run in RunSet Mode when all protocols within the same staining run follow the exact same RunSet pattern. Therefore, it is important to ensure that all protocol templates and special protocols conform to the following requirements:

a. The number of Steps in Set A, B and C must be fixed between protocols.
b. The number of Steps in the last Set, D, may vary between protocols (minimum 1 step).
   - *Note: If it is necessary to add additional steps and Sets, please ensure that only the last Set contains a variable number of step ie: AAABBCDDDEEFFFF(G variable).*
   - *Note: System Limitation. The scheduling process may timeout for complex protocols with too many Sets or too many pooled Groups. The run scheduler employs simulation within each Set, to generate the most efficient runtime schedule; therefore, increasing the number of Sets or Groups will result in a longer scheduling calculation time.*

ISH protocols, Double Stain and Special protocols may be run together with IHC protocols in RunSet Mode; however, the protocol Sets must be configured to follow the same RunSet pattern as the standard IHC protocols. The number of steps in Set D can be increased or reduced to conform to the standard RunSet pattern.

a. AA, for first and second Dewax Step
b. BB, for third and fourth Dewax Step
c. C, for final Dewax Step
d. DDDDDDDDDDDD, all remaining Steps



	Step Index	Set	Extract	Link	Max Group Size	Reagent Type	Reagent Name	Volume (µL)	Incubation Type	Incubation Time	Incubation Temperature Table	Agitation	Wash Buffer Type	Wash Buffer	#	Volume (µL)	Edit Mask
▸	1	A	☐	☐	18	Dewax	DS3	130	Normal	00:06:00	00:00:00 - 00:30:00 65°C	Inject 00:00:01 A7 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
	2	A	☑	☐	18	Dewax	DS3	130	Normal	00:06:00	00:00:00 - 00:30:00 62°C	Inject 00:00:01 A7 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
	3	B	☑	☐	18	Dewax	DS3	130	Normal	00:06:00	00:00:00 - 00:30:00 62°C	Inject 00:00:01 A7 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
	4	B	☑	☐	18	Dewax	DS3	130	Normal	00:06:00	00:00:00 - 00:30:00 62°C	Inject 00:00:01 A7 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
	5	C	☑	☐	18	Dewax	DS3	130	Normal	00:06:00	00:00:00 - 00:30:00 62°C	Inject 00:00:01 A7 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
	6	D	☑	☐	18	Dewax	DS4	130	Normal	00:06:00	00:00:00 - 00:59:00 50°C	Inject 00:00:01 A7 00:00:59 T < 71°C	Buffer	System Fluid	1	220	0
	7	D	☑	☐	18	Retrieval	TR3, ISH	340	Normal	00:22:00	00:00:00 - 00:15:00 98°C 00:15:00 - 00:20:00 65°C	A3 00:11:00 A4 00:11:00 T < 110°C	Buffer	System Fluid	1	200	0
	8	D	☑	☐	18	Buffer	System Fluid	130	Normal	00:06:00	00:00:00 - 00:59:00 40°C	Inject 00:00:01 A7 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
	9	D	☑	☐	18	Buffer	System Fluid	130	Normal	00:06:00	00:00:00 - 00:59:00 40°C	Inject 00:00:01 A7 00:00:59 T < 71°C	Buffer	System Fluid	0	85	0
	10	D	☑	☐	18	Buffer	System Fluid	1	Normal	00:20:00	00:00:00 - 00:15:00 45°C	Extract 00:02:00 Down 00:00:06 T < 71°C	Buffer	System Fluid	0	85	0
	11	D	☐	☐	18	Detect	Pepsin	130	Normal	00:30:00	00:00:00 - 00:30:00 37°C	Inject 00:00:01 A7 00:04:59 T < 71°C	Buffer	System Fluid	2	220	0

Figure 42

Dynamic Mode

This mode of scheduling may result in longer runtimes as the system will run each protocol individually.

The system will run in Dynamic Mode when the system detects protocols with incongruent RunSet patterns.
Note: Additionally, the system will run in Dynamic Mode to minimize overall runtime for a group of 1 slide.

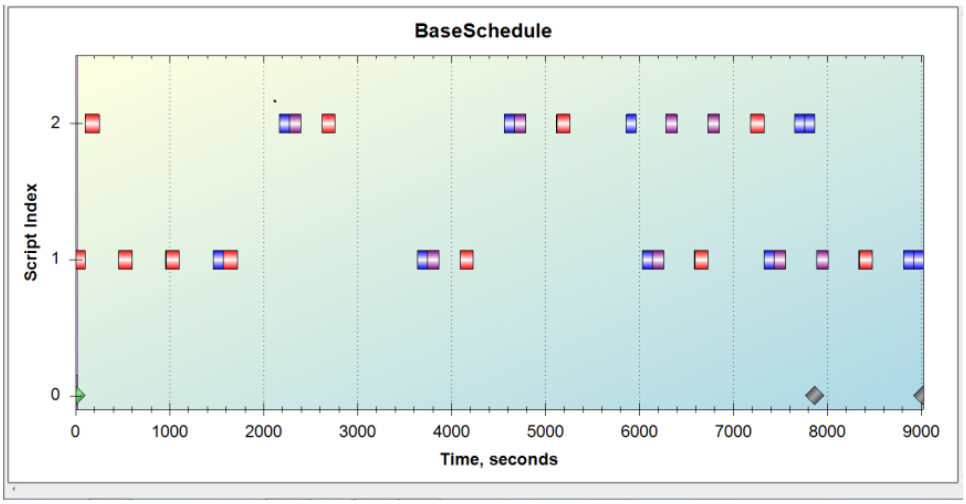


Figure 43

Runtime Optimization

It is important to consider the overall runtime when constructing protocol templates.

a. Protocols with many steps will take more time to execute; therefore, to improve the base runtime:
   - Minimize the number of protocol Steps and Quick Washes.
   - Minimize the number of extractions; set no extraction on the first Step (after baking).
   - Reduce incubation times.
   - Minimize the use of on-rack mixing or on-board mixing reagents.

b. Protocols with many time-sensitive steps will be more difficult to schedule to run in tandem with other protocols; therefore, to improve scheduling flexibility:
   - Minimize the number of Quick Washes and Linked Steps.
   - Setup an Incubated Wash Step if the reagent incubation is not time-sensitive.
   - Maximize the Pooled group size. *Note: Set the Max Group Size and ensure that the reagent volume can be aspirated and delivered in one draw. The aspiration volume for a group of 18 slides is < 5mL.*
   - Optimize incubation times. *Note: It is generally recommended to set incubation times >5min to allot sufficient idle time for another process to run in tandem. Perform run simulations to determine which incubation steps may be modified to reduce overall runtime.*

c. Protocols with few common steps will generate more complex runs; therefore, to maximize scheduling efficiency:
   - Match the RunSet pattern across protocol templates and special protocols.
   - Maximize the number of common Steps.
   - Optimize the number of Sets. *Note: Perform schedule simulations to determine which Steps may be grouped into a Set to structure the run more efficiently.*
   - Minimize the use of different protocol customizations.
   - Minimize the use of different protocol templates and special protocols (esp. within the same run).

Note: There is an expected delay between calculated runtime and actual runtime. The system is not 100% efficient and there will be some variability in the time to execute the processes in the real staining run. Therefore, the scheduler will slightly "under-schedule" the time required to complete each task to create the most efficient schedule and minimize the overall runtime. Expect 0- 1.5 hours delay, based on the size and complexity of the run.

Section 4: Creating Reagents

The PCStainer's Reagent Editor provides specialists the basic tools to add/remove/edit reagents. *Refer to the Operating Manual for further details.*

Click  and login to the application using a Distributor-level account.
Default User ID: Distributor
Default Password: Distributor

4.1 Reagents

Overview of Reagents

Buffer

Block is a hydrogen peroxidase blocking solution.

BS buffer is a Tris-EDTA-based bridge solution applied before the heat pretreatment step.

System Fluid (for IHC and ISH)/Buffer is a TBS-based wash buffer pH7.4 with Tween 20.

Dewax
DS1 (for IHC) and DS3 (for ISH) are organic-solvent-based solutions used to remove paraffin wax.

DS4 is a pretreatment solution formulated for ISH.

DS2-50 is an organic-solvent-based solution used to remove the remaining wax residues following dewaxing and assists in tissue pretreatment to enhance staining intensity.

DSE-50 (for IHC) is a pepsin-based solution used for tissue pretreatment.

Retrieval
TR1 is a pH9.0 Tris-EDTA-based target retrieval solution with peroxidase blocking function.

TR2 is a pH6.0 citrate -based target retrieval solution with weaker peroxidase blocking function than TR1.

TR3 is a target retrieval solution formulated for ISH.

TR4 is a pH9.0 Tris-EDTA-based target retrieval solution with enhanced retrieval capability.

AP, AP Plus, AP Super, APx, APx Plus
All RTU antibodies assigned to AP Protocol Types

HRP, HRP Plus, HRP Super, HRPx, HRPx Plus
All RTU antibodies assigned to HRP Protocol Types

ISH, ISH HRP Super, ISH AP Super
All RTU probes assigned to ISH Protocol Types

FISH, FISH Plus
All RTU probes assigned to FISH Protocol Types

Negative
All negative control solutions.

Detect
AP Polymer is a 1-step AP Polymer solution.

Polymer Enhancer and AP Polymer-2 are the components of the 2-step AP Polymer detection system.
HRP Polymer is a 1-step HRP Polymer solution.

Red on Board (Red Substrate, Red Chromogen) is a 1:1 Fast Red chromogenic solution mixed directly on the slide.

Polymer Enhancer and HRP Polymer-2 are the components of the 2-step HRP Polymer detection system.

HRP-A, HRP-B, HRP-C are the components of the 3-step HRP Polymer detection system.

DAB on Board (DAB Substrate, DAB Chromogen) is a 1:1 DAB chromogenic solution mixed directly on the slide.

Others
DS Buffer and TR Buffer (same as System Fluid)

TR Enzyme (for IHC), DS Enzyme (for CISH) and Pepsin (for ISH) are pepsin-based solutions used for tissue pretreatment.
Note: The standard pepsin concentration is 2.5mg/ml.

Hematoxylin is a nuclear counterstain stain solution.

Special
User's custom reagents

Creating a New Reagent

The system is preloaded with a list of reagents, categorized by Reagent Type.
Note: Certain Reagent Types such as Dewax and Retrieval may not be available to the user. Contact Technical Support for more information.

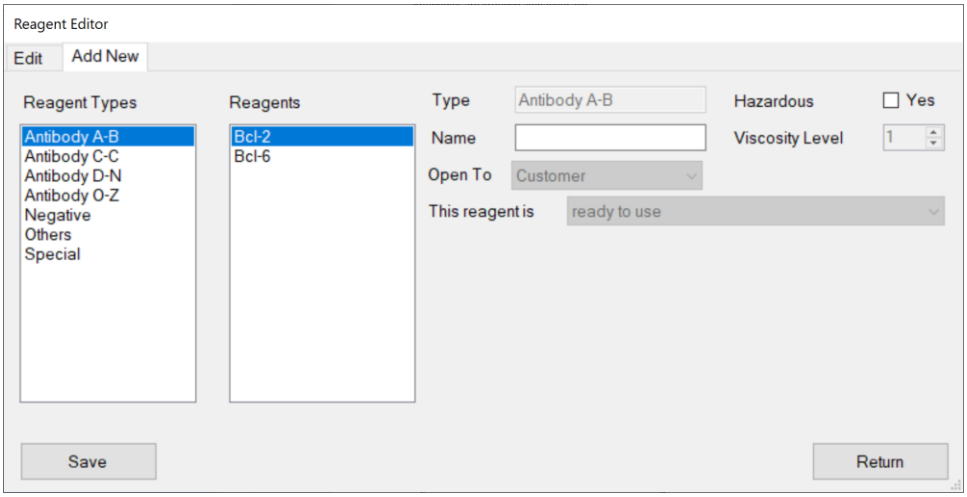


Figure 44

To add a new reagent, open System Utilities>Editors>**Reagent Editor**.

a. Select the tab, Add New.
b. Select the appropriate Reagent Type.
c. Enter the Name.
   Note: Each reagent (from different source/different clone) must have a unique reagent name.
d. Select the Hazardous Status, if applicable.
e. Select the Viscosity Level, if applicable.
f. Select the security access level, Open To.
g. Select the Reagent Mixing Parameters.
h. Click "**Save**".

Deleting a Reagent

To delete an existing reagent, open System Utilities>Editors>**Reagent Editor**.

a. Select the tab, Edit.
b. Select the appropriate Reagent Type and Reagent from the list.
c. Click "**Delete**".
   Note1: The reagent cannot be deleted if it is used in an existing protocol on the system.
   Note2: The reagent cannot be deleted if it is above the user's security access level.

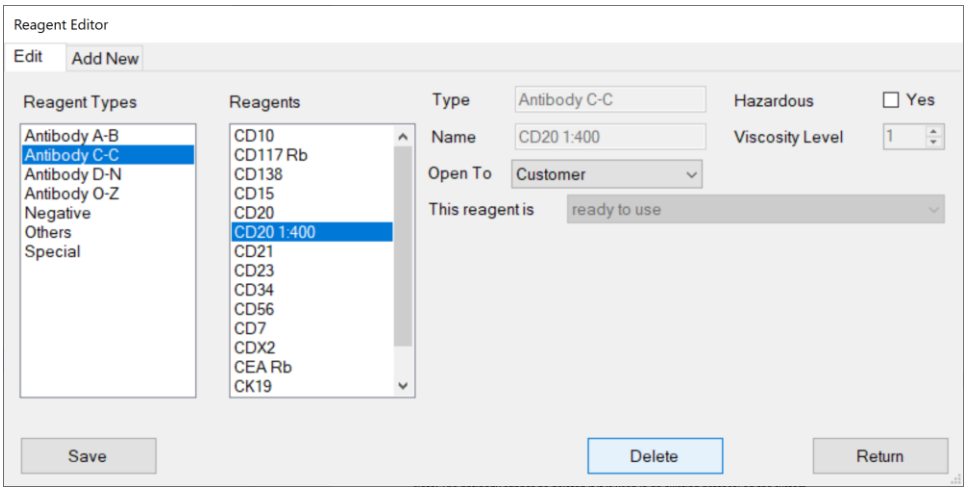


Figure 45

Modifying an Existing Reagent

To modify the properties of an existing reagent, open System Utilities>Editors>**Reagent Editor**.

a. Select the tab, Edit.
b. Select the appropriate Reagent Type and Reagent from the list.
c. Modify the reagent properties.
   Note: Some reagent properties may not be available, based on the user's security access level.
d. Click "**Save**".

4.2 Reagent Properties

Each reagent has several properties that can be configured for its waste management, mixing ratios, and unique liquid handling requirements.

Reagent Hazardous Status

Check the box **Yes** to indicate the reagent is Hazardous.
All hazardous reagents will be dispensed into the hazardous waste station to facilitate proper waste management.
Note1: Probe1 rinse/wash after dispensation of hazardous reagent will be deemed hazardous.
Note2: Probe2 rinse after extraction of hazardous reagent waste will be deemed hazardous.
Note3: Probe2 rinse after extraction of succeeding (wash) step after hazardous reagent step will be deemed hazardous.

Reagent Viscosity Levels

Select the reagent's Viscosity Level from the dropdown list.
The system will use the Viscosity Level to determine the special liquid handling features required for the reagent.
The reagent aspiration and dispensation are optimized for each viscosity level.
Note: Generally, select the default viscosity level 1. Otherwise, select the same level as a comparable reagent.

1) Viscosity 1: Buffer-based reagents; Abs, Polymers, enzymes, Hematoxylin, etc.
   a. Minimize draw factor.
2) Viscosity 2: Dewax solutions
   a. A certain volume of liquid affected by inner-tubing dilution will be discarded.
3) Viscosity 3: Retrieval solutions (pH-sensitive)
   a. A certain volume of liquid affected by inner-tubing dilution will be discarded.
4) Viscosity 4: Probes (high viscosity)
5) Viscosity 5 (TBD)
6) Viscosity 6: Buffers and wash solution

Security

Select the reagent's security access level from the dropdown list, Open To.
This feature may be used to safeguard essential system reagents from modification or deletion.

Certain features of the Reagent Editor and RFID Editor are restricted based on the user's security access level.

Feature	Distributor	Supervisor
Create an antibody	✓	✓
Create Special, Other, Negative	✓	✓
Create Dewax, Retrieval, Detection and other Reagent Types	✓*	×
Delete a reagent	Except reagents Open To "Closed"	Only reagents Open To "Customer"
Edit a reagent	Except reagents Open To "Closed"	Only reagents Open To "Customer"
Create a new reagent type	×	×
Program/re-program RFID tag	✓*	Only reagents Open To "Customer"

* *Note: Some Reagent Types may be restricted by the manufacturer and are not displayed in the Reagent Editor or RFID Editor.*

Reagent Mixing Parameters

The system provides several options for on-slide and on-rack mixing of reagents to limit the user's direct contact with hazardous reagents and accommodate protocols which require unstable/perishable working solutions.

1) **Ready-to-use**. Select this option for pre-mixed and RTU solutions.

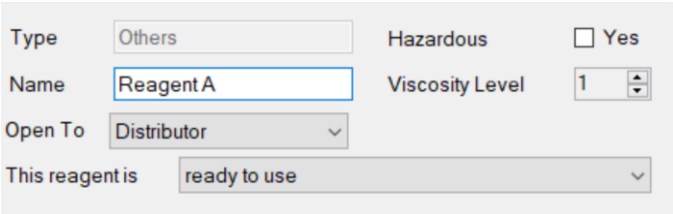


Figure 46

2) **Mixed on-board (on-slide)**. Select this option for 2-component working solutions which can be applied and mixed directly on the slide, such as those with a low mixing ratio, ie: 1:1.
Note: Reagent mixtures that have a short activity time must be mixed on-board.

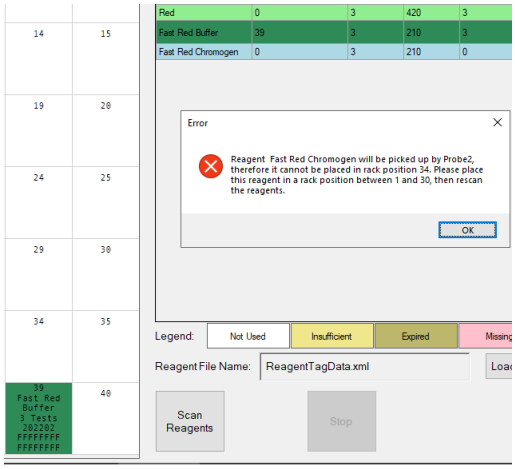


Figure 47

The software will designate the reagent dispensation probe Z1 to aspirate the first mixing component and the waste extraction probe Z2 to aspirate the second mixing component. The reagent mixture will be prepared on each slide on the step in which the mixture is used. The user should verify that the working solution has sufficient mixing accuracy and homogeneity.
Note: Increase the test volume to 220uL per slide, as needed.

a. To create an on-board mixing reagent, open System Utilities>Editors>**Reagent Editor**.
   - Add a new reagent for Reagent A as ready-to-use.
     Note: Reagent A may contact the tissue for a brief time. It is important to confirm that Reagent A will not negatively affect staining as a single component. If staining uniformity is an issue, the user may need to switch the order of Reagent A and Reagent B or implement other methods of mixing.
   - Add a new reagent for Reagent B as ready-to-use.
   - Add a new reagent for the mixed reagent solution ie: Reagent AB
   - Set the Ratio of Reagent A: Reagent B for the mixture.
     Note: It is recommended that the reagents have a relatively even ratio 1:1, to ensure dispensation accuracy. Recommended minimum dispensation volume is 65uL per slide for each component. Reformulate the component solutions or implement a different mixing method, as needed.
   - Click "**Save**".

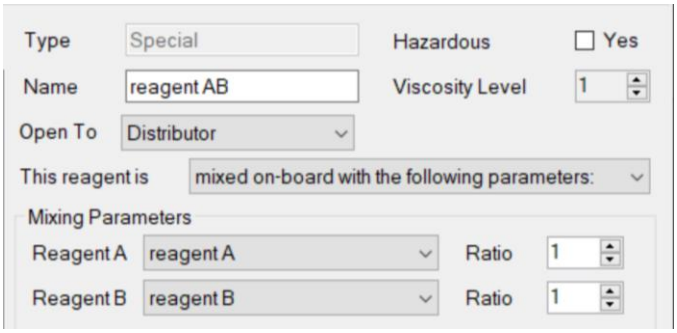


Figure 48

b. **Mechanism of Action for on-board mixing:**
Probe 2 will extract reagent waste from the previous Step for all slides in the group. Probe 1 will aspirate Reagent A and Probe 2 will aspirate Reagent B for all slides in the group. For each slide, Probe 1 will dispense Reagent A, followed immediately by Probe 2 dispensing Reagent B, before moving to the next slide in the group. Immediately after dispensation by Probe 1 and 2, each module lid will perform several quick agitations to mix the reagents evenly.

3) **Mixed on-rack**. Select this option for 2 or 3-component working solutions which must be mixed in an empty vial on the reagent rack, such as those with a high mixing ratio, ie: 1:30.
Note: The maximum mixture volume must not exceed 16mL.

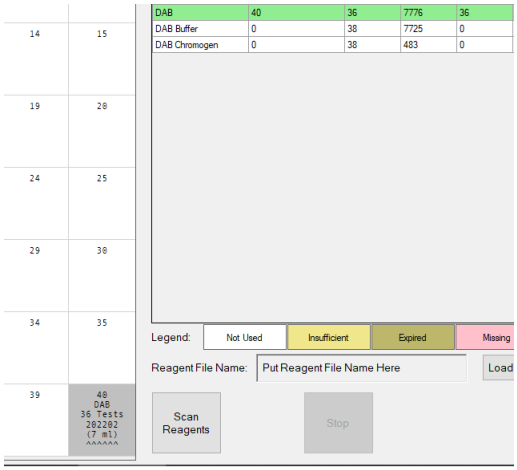


Figure 49

The software will designate one position on the reagent rack to place an empty 7mL or 15mL vial for mixing, ie: position #40. The bottle size will depend on the total mixing volume required. The reagent mixture will be prepared immediately prior to the first step in which the mixture is used. The user should verify that the working solution can retain sufficient activity if it is applied later in subsequent steps and/or if it is applied to other groups of slides.
Note: On-rack mixing is less resource-efficient than other mixing methods. A few additional tests of each component must be consumed per mixture to ensure there is sufficient dead volume in the mixing vial and to meet the minimum mixing volume requirement.

a. To create an on-rack mixing reagent, open System Utilities>Editors>**Reagent Editor**.
   - Add a new reagent for Reagent A as ready-to-use.
   - Add a new reagent for Reagent B as ready-to-use.
   - Add a new reagent for Reagent C (if applicable) as ready-to-use.
   - Add a new reagent for the mixed reagent solution ie: Reagent AB or Reagent ABC
   - Select the option "mixed on-rack…"
   - Set the Ratio of Reagent A: Reagent B: Reagent C for the mixture.
     Note: Ensure that the reagent with the smallest ratio term goes first.
   - Set the **Minimum Volume*** to ensure accurate mixing.
     Note: Reagent A, B (and C optional) may have a high mixing ratio, ie: 1:50; however, the user must set an appropriate minimum mixing volume to ensure the compositional accuracy of the mixture.
   - Click "**Save**".
   - *Note: Solutions mixed on-rack may be more significantly affected by inner tubing dilution. The user should verify the mixture's activity in comparison to a pre-mixed solution.*

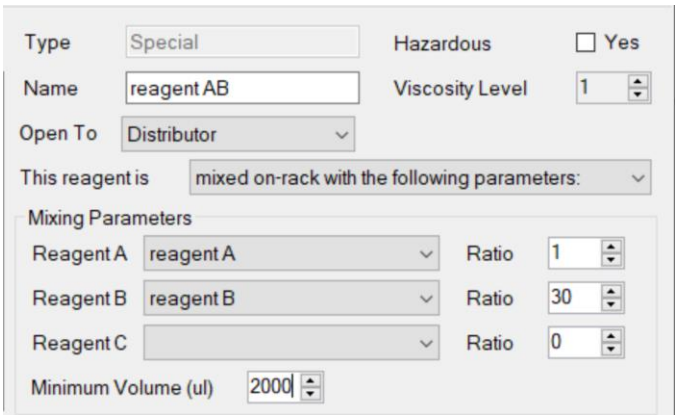


Figure 50

b. **Mechanism of Action for 2-component on-rack mixing:**
Probe 1 will aspirate Reagent A and dispense the solution into the empty reagent vial, then wash/rinse the probe. Probe 1 will aspirate Reagent B and dispense the solution into the vial and mix the solutions by aspiration, then immediately aspirate the mixture for all slides in the group. For each slide, Probe 2 will extract waste, followed immediately by Probe 1 dispensing the reagent mixture before moving to the next slide in the group. Immediately after dispensation by Probe 2, each module lid will move to the lower lid position.

c. **Mechanism of Action for 3-component on-rack mixing:**
Probe 1 will aspirate Reagent A and dispense the solution into the empty reagent vial, then wash/rinse the probe. Probe 1 will aspirate Reagent B and dispense the solution into the vial, then wash/rinse the probe. Probe 1 will aspirate Reagent C and dispense the solution into the vial and mix the solutions by aspiration, then immediately aspirate the mixture for all slides in the group. For each slide, Probe 2 will extract waste, followed immediately by Probe 1 dispensing the reagent mixture before moving to the next slide in the group. Immediately after dispensation by Probe 2, each module lid will move to the lower lid position.

d. ***Calculation for Minimum Volume**
   - Divide each term of the Ratio by the smallest term.
   - Find the sum of the terms.
   - Multiply by 65uL (recommended minimum dispensation volume).
   - *For example: Reagent A:B:C is mixed in a ratio of **2:3:4**. Divide each term by **2** to derive the ratio **1:1.5:2**. Find the sum the terms **4.5** to derive the volume **292.5uL**. Round to **300uL**.*

Special Liquid Handling Features

a. Reagent Name: System Fluid, DS Buffer, TR Buffer, and Buffer
   - Reagent volume is drawn from the Wash Buffer bottle through the in-line tubing.

b. Viscosity Level 3
   - This feature is optimized for solutions significantly affected by inner tubing dilution, such as TR.
   - The reagent is delivered in two dispensations:
     1) First dispense: ½ the required test volume is aspirated and dispensed to the group of slides in ascending order.
     2) Second dispense: ½ the required test volume is aspirated and dispensed to the group of slides in descending order.

c. Viscosity Level 4
   - Reagent is independently aspirated and dispensed to each slide individually.
   - The aspiration delay time is longer, optimized for viscous probe solutions.
   - The pump dispense speed is slower, optimized for viscous probe solutions.

d. Incubation Type: Fine
   - Reagent is independently aspirated, dispensed and incubated for an exact time on each slide individually. After waste extraction, System Fluid is immediately applied to stop further incubation on the slide.

e. Incubation Type: AirDry
   - The reagent is independently aspirated, dispensed and incubated for an exact time on each slide individually. After waste extraction, the system will not apply System Fluid and allow the slide to dry.

f. Reagent Name: DAlcohol
   - The reagent, DAlcohol, is independently aspirated, dispensed and incubated for an exact time on each slide individually. After waste extraction, the system will not apply System Fluid and allow the slide to dry.

g. Others
   - Customize “waiting” and “shaking” time in the GP Editor after reagent aspiration based on viscosity level.
   - Customize extra probe wash cycles in the GP Editor after reagent dispensation based on Reagent Name or Reagent Type.
   - (Configured by manufacturer) Custom agitation/dispensation to minimize exposure to air.
   - (Configured by manufacturer) Enable agitation after dispensation to improve mixing.
   - (Configured by manufacturer) Enable temperature monitoring during injection.
   - *Contact Technical Support for more information.*

Vialing Volumes

Overview

The system is designed for use with two sizes of reagent vial:

1) **7mL size**, capacity 9mL, recommended for:
    a. Antibodies, probes
    b. Enzymes and other reagents that are not stable at RT for prolonged periods
    c. 2-component DAB 1:1, 2-component Red 1:1 (110 uL per test per component)
    d. Pre-mixed working solutions
2) **15mL size**, capacity 16mL, recommended for:
    a. Dewax solutions
    b. TR solutions and pretreatment ancillaries
    c. Polymers
    d. DI Water, alcohol, buffers and wash solutions

The vials are molded from chemical-resistant HDPE with two color options:

1) **Clear-white**
2) **Opaque-black**, recommended for light-sensitive reagents, ie: fluorescent probes, chromogens, dyes, etc.

Note: The vial caps are molded from PP with a foam liner to prevent leaking.

The vials are designed with a recess on the top surface, to facilitate RFID tagging.

1) **Option with RFID tag**, recommended for tracking reagent tests in a test kit
2) **Option without RFID tag**, recommended for disposable/refillable vial option.

Standard Test Volume

The system is calibrated for use with either the small volume or large volume chambers.
The **standard test volume** for one test is:

1) **130uL** for **100uL chambers,** recommended for minimizing reagent volumes/waste.
2) **200uL** for **200uL chambers**, recommended for achieving a more stable, uniform staining area.
    Examples: applications that require long incubations or frequent washes, Special Stains

Note1: Test volume refers to the volume set in the protocol.
Note2: The 100uL chambers may be set to a higher lid angle to accommodate larger volumes of liquid, ie: 240uL wash at A4; however, doing so may increase the chance of reagent leaking from the slide.

Tip: The volume of the chamber is embossed on its surface.
Marked as "85uL" for 100uL chambers and "150uL" for 200uL chambers.

Draw Factor

The user should account for the draw factor when determining the vialing volume for a reagent.
The system must draw an extra volume of reagent to ensure accurate dispensation.
Therefore, the test volume must be augmented by a draw factor: **The standard draw factor is 1.08**.

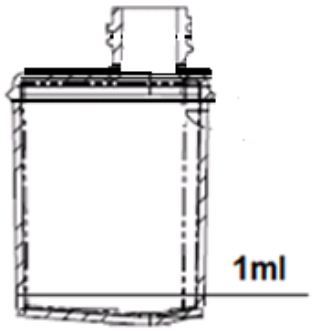
Dispense Factor

The user should account for the dispense factor when determining the test volume.
The system may adjust the dispensation volume to improve consistency based on the reagent's liquid properties. Additionally, a small portion of the aspirated reagent may be discarded to reduce the effects of inner tubing dilution. **The standard dispense factor is 1**; however, in some cases, it may be <1.

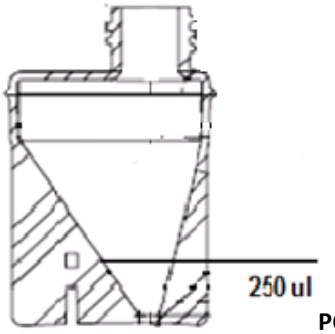
Dead Volume

The **dead volume** for each vial type is:

1) **250uL/500uL** for the **7mL vial (130uL/200uL standard test volume)**
2) **1000uL** for **15mL vials**



PC026, 15ml



PC085, 7ml

Minimum Volume

The **minimum volume** for one standard test in a 7mL vial is:

1) For 100uL chambers: **130uL x 1.08 + 250uL = 390uL**
2) For 200uL chambers: **200uL x 1.08 + 500uL = 716uL**

Calculated Vialing Volume

The **vialing volume** for ***n*** number of standard tests in a 7mL vial is:

1) For 100uL chambers: **130uL x 1.08 x *n* + 250uL**
2) For 200uL chambers: **200uL x 1.08 x *n* + 500uL**

The **vialing volume** for ***n*** number of standard tests in a 15mL vial is:

1) For 100uL chambers: **130uL x 1.08 x *n* + 1000uL**
2) For 200uL chambers: **200uL x 1.08 x *n* + 1000uL**

*Tip: To determine the total required reagent volumes for a staining run, assign the protocols and click "**Scan Reagents**" to load the Reagent List. This list will display the volume required (x1.08 draw factor) for each reagent. Divide the volume into the appropriate number/type of vial(s) and add the dead volume to each vial.*

Actual Vialing Volume

The actual vialing volume required may be affected based on the test volume, tests per vial and the reagent's unique liquid properties. Real-time testing should be performed to determine optimal volumes.

1) Some reagents require a larger test volume
a. Example: Dewax solutions, 220-240uL
b. Example: TR solutions, 360-400uL
2) Some high viscosity reagents may adhere to the outer surface of the probe and vial, requiring extra volume.
3) Some reagents have a higher rate of evaporation, requiring extra volume
4) Some reagents (perishable or higher rate of evaporation) may be vialed with fewer tests per vial.
    a. Example: Pepsin, 10 tests per vial

Recommended Vialing Volumes

1) **Viscosity Group #1:** Abs, Detections, and other buffer-based reagents…

Recommended Volumes for most Buffer-based Reagents*				
130ul chamber	10 Tests	25 Tests	50 Tests	100 Tests
15ml vial	2.50ml	4.50ml	8.00ml	15.00ml
7ml vial	1.60ml	3.75ml	7.25ml	

**recommendation based on 130uL test volume*

Recommended Volumes for DAB 1:1/Red 1:1 Substrate and Chromogen*				
130ul chamber	10 Tests	25 Tests	50 Tests	100 Tests
15ml vial	2.25ml	4.00ml	7.00ml	13.50ml
7ml vial	1.50ml	3.25ml	6.25ml	

**recommendation based on 110uL test volume for 1:1 on-board mixing*

Recommended Volumes for TR Enzyme*			
130ul chamber	10 Tests	20 tests	25 Tests
PC026, 15ml vial		13.25ml	16.00ml
PC085, 7ml vial	6.50ml		

**recommendation based on 540uL test volume*

Recommended Volumes for TR Enzyme**			
130ul chamber	10 Tests	20 tests	34 Tests
PC026, 15ml vial		9.75ml	15.50ml
PC085, 7ml vial	4.75ml	9.00ml	

***recommendation based on 370uL test volume*

2) **Viscosity Group#2**: Dewax Solutions 1, 2, 3 and 4

Recommended Volumes for Dewax Solutions*			
130ul chamber	40 Tests	50 Tests	100 Tests
PC026, 15ml vial	7.00ml	8.50ml	16.00ml
PC085, 7ml vial	6.25ml	7.75ml	

**recommendation based on 130uL test volume*

Recommended Volumes for Dewax Solutions**			
130ul chamber	40 tests	50 Tests	100 Tests
PC026, 15ml vial	12.50ml	15.00ml	

***recommendation based on 240uL test volume*

3) **Viscosity Group#3:** Retrieval Solutions TR1, TR2, TR3, TR4

Recommended Volumes for Retrieval Solutions*			
130ul chamber	10 Tests	20 tests	25 Tests
PC026, 15ml vial		13.25ml	16.00ml
PC085, 7ml vial	6.50ml		

**recommendation based on 540uL test volume*

Recommended Volumes for Retrieval Solutions**			
130ul chamber	10 Tests	20 tests	34 Tests
PC026, 15ml vial		9.75ml	15.50ml
PC085, 7ml vial	4.75ml	9.00ml	

***recommendation based on 370uL test volume*

Recommended Volumes for FISH/CISH Retrieval Solution*		
130ul chamber	40 Tests	
PC026, 15ml vial only	16.00ml	

**recommendation based on 340uL test volume (smaller staining area)*

4) **Viscosity Group#4:** FISH and/or CISH Probes based on ZV probe diluent (g/ml 1:1.24)

Recommended Volumes for FISH/CISH Probes*		
130ul chamber	10 Tests	20 Tests
PC085, 7ml vial only	1.60ml	2.90ml
Approx. weight	2.00g	3.60g

**recommendation based on 130uL test volume*

5) **Viscosity Group#5:** Reserved for new reagent type with different viscosities (TBD)

6) **Viscosity Group#6:** System Fluid, Other Buffer, DI Water, TR Buffer, DS Buffer, SSC Solution

Recommended Volume for most Buffer/Wash Solutions (same as group#1)*				
130ul chamber	10 Tests	25 Tests	50 Tests	100 Tests
PC026, 15ml vial	2.50ml	4.50ml	8.00ml	15.00ml
PC085, 7ml vial	1.60ml	3.75ml	7.25ml	

**recommendation based on130uL test volume*

Recommended Volume for most Buffer/Wash Solutions (same as group#1)*				
130ul chamber	10 Tests	25 Tests	50 Tests	100 Tests
PC026, 15ml vial	3.50ml	7.50ml	14.00ml	
PC085, 7ml vial	2.80ml	6.75ml	13.25ml	

***recommendation based on240uL test volume*

4.3 Reagent Tracking

The reagents' RFID tag information (remaining test number, lot number, expiration and manufacturer) is registered and tracked in the system by each RFID tag's unique ID:

Scan Reagents

During reagent scanning, the system will verify the data stored in each RFID tag. The user will receive a warning if:

a. The number of tests is insufficient to complete the staining run.
b. The number of tests recorded in the RFID tag does not match the number of tests registered in the system's reagent tracking database.
c. The reagents are expired.

Staining Process

During the run, the system will register any new RFID tags and update the remaining test number.

RFID Editor

During RFID programming, the user can re-register old RFID tags to re-use vials or update RFID tag information.
Refer to the Operating Manual for more information.

4.4 Titration

The user may optimize antibody dilution by testing multiple antibody titers on the system.

Use the Reagent Editor to add multiple antibody titers to the system.
The reagent name should contain the antibody name and dilution.

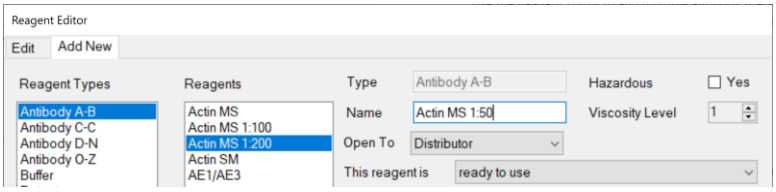


Figure 51

Use the Protocol Editor to add the protocol for each antibody titer to match original staining conditions.

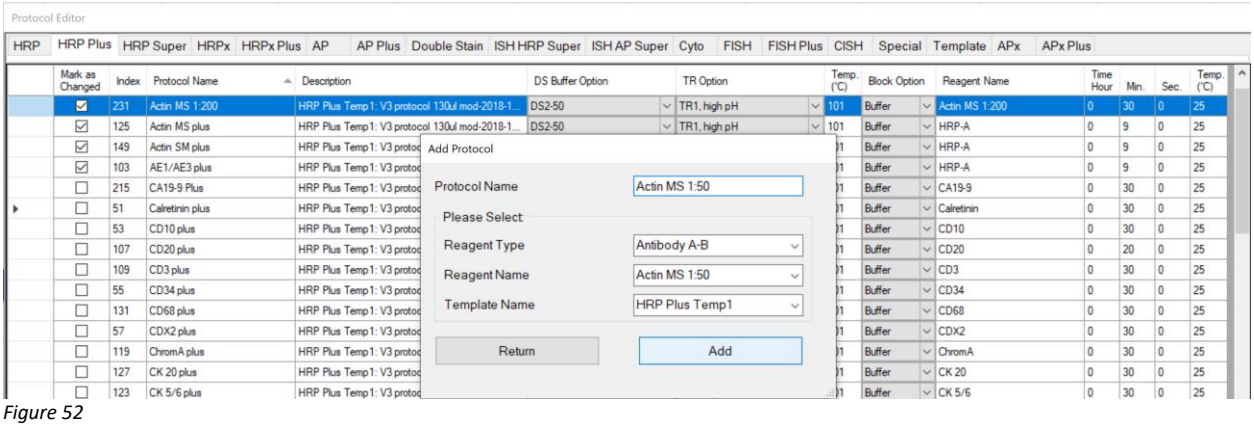


Figure 52

Use a disposable 7mL vial to prepare each antibody titer and manually assign the antibody titers.
Run the antibody titers in a routine staining run and verify the staining results.
Delete the antibody titers from the Reagent Editor and Protocol Editor after testing is completed.

Section 5: Distributing Protocols

The Protocol Reagent Manager provides specialists the basic tools to transfer protocols and reagents between systems.

Click  and login to the application using a Distributor-level account.
Default User ID: Distributor
Default Password: Distributor

5.1 Protocol and Reagent Files

The system's protocols and reagents are stored in the key system data files located in the Local Disk C:\PathCom\PathComASGui\Data

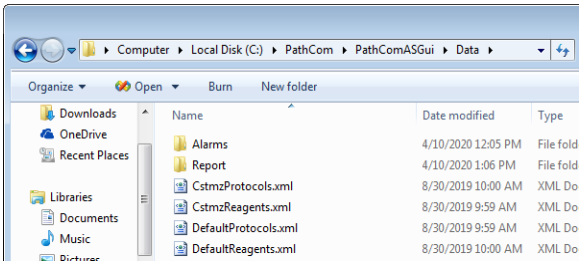


Figure 53

a. The current "working" set of protocols and reagents is stored in the files:
   - **Protocols.xml**
   - **Reagents.xml**
b. The latest saved set of protocol customizations (and accompanying reagents) is stored in the files:
   - **CstmzProtocols.xml**
   - **CstmzReagents.xml**
c. The latest set of manufacturer's default protocols and reagents is stored in the files:
   - **DefaultProtocols.xml**
   - **DefaultReagents.xml**
d. The negative control links (assigned negative control reagent) for all protocols are stored in the file:
   - **NegativeControl.xml**
e. The latest configuration of selected protocol templates (in Change Protocol Template) are stored in the file:
   - **templatecfg.dat**

These files may be copied and directly transferred to the user to update all templates, protocols, and reagents. However, this method may not be convenient for routine use, as the user's protocols and reagents will be completely overwritten and the transfer file sizes are very large.

Use the Protocol Reagent Manager to manage protocols and reagents more precisely.

5.2 Export Package

The user may export select templates, protocols, and reagents to a compressed zipped folder to be shared with other systems.

Creating an Export Package

Note: Before creating an Export package, please verify the protocols, assigned negative controls, and reagents, then save all protocol customizations using System Utilities>Settings>"Save All Customizations".

To create an export package, open System Utilities>Editors>**Protocol Reagent Manager**

a. Login to the application. *Default User ID: Distributor, Default Password: Distributor*
   Note: Only Distributor-level users may export protocols.
b. Click "**Browse**" in the field, **From Folder**, and select the folder containing the Protocols.xml and Reagents.xml.
c. Click "**Browse**" in the field, **Export Folder**, and select/create a new export folder.
d. Enter a filename in the field for **Export File Name**.
e. Select protocols and/or templates under **Available Protocols to Export**.
   - Select a Protocol Type, Special or Template from the dropdown to view the corresponding list of protocols.
   - *Tip: Press the Ctrl key to select multiple protocols.*
   - Note: If selecting a standard protocol, it may be necessary to include its assigned protocol template if it does not exist on the importing system.

d. Click "**Add**" to add the protocols to the list of **Protocols to Export**.
   - The associated reagent(s) to be exported are listed under **Reagents to Export**.
   - The associated negative control links) to be exported are listed under **Negative Controls to Export**.

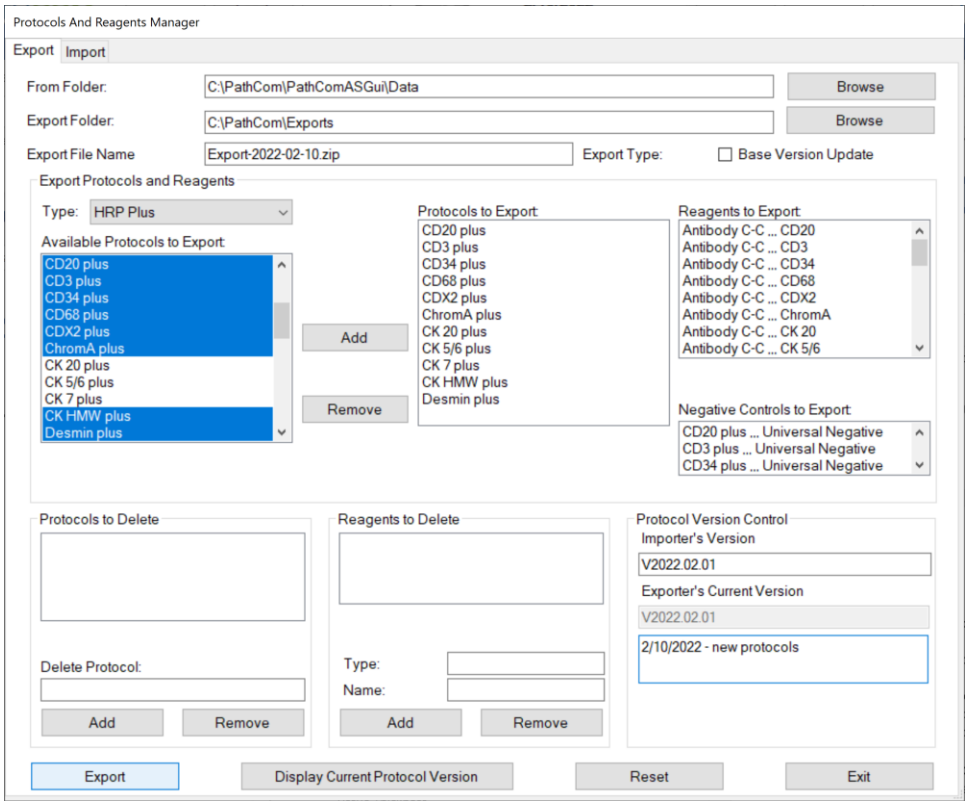


Figure 54

- Select the Base Version Update option, as needed. *Refer to Protocol Version Control for more information.*
- The Exporter's Current Version is displayed by default.
- Enter a description in the box below, next to the date.

e. Click "**Export**" to export the selected protocols and associated reagents, negative controls and saved customizations to a compressed zip folder.
f. The system will display a confirmation message "Finished' when the process is completed.
g. Click "**Exit**" to close the utility.

Transferring an Export Package

To transfer an export package electronically to another system, open the Export folder.

a. Copy the Export Package from the assigned file location, ie: C:\PathCom\Exports
   - *Note: Export packages stored in the default C:\ PathCom\Exports folder will be deleted automatically whenever the SW is updated.*

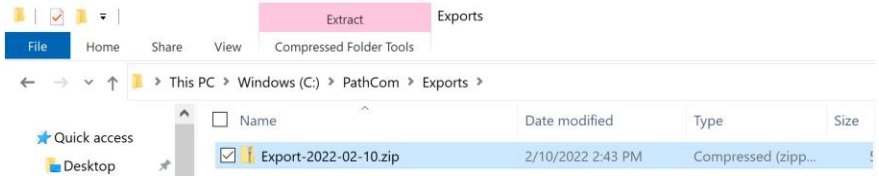


Figure 55

b. Distribute the Export Package by email, external USB flash drive or online file-sharing service.
c. The importing computer must download the Export Package to a location on the Local Disk C:\ or the Desktop.
d. Confirm the SW versions on both systems are compatible and contain the latest Protocol Types.
   - *Note: To avoid conflicts, the exporter and importer's systems must be running the same SW branch (and preferably the same SW version).*

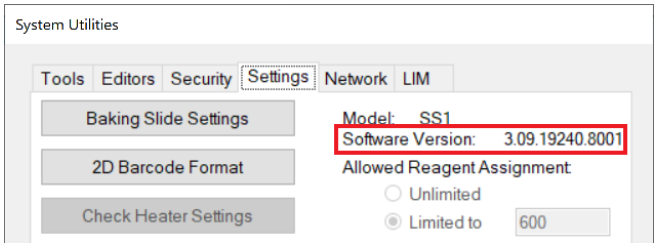


Figure 56

Importing an Export Package

Note: Before importing an Export Package, save all current customizations and create a backup of the current protocol and reagent files. The system will refresh ALL protocols at the end of the import process. All unsaved changes will be overwritten with the previously saved customizations.

To import an export package, open System Utilities>Editors>**Protocol Reagent Manager**

a. Login to the application. *Default User ID: Distributor, Default Password: Distributor*
   Note: Supervisor-level and Distributor-level users may import protocols.
b. Click "**Browse**" in the field for **Import File** and select an export package (compressed zip folder).

   ⚠ **Do not browse directly to an external USB flash drive or Network folder. Always copy the package to a location on the Local Disk C:\ or the Desktop.**

   - The protocols to be imported are listed under Import Protocols.
   - The associated reagent(s) to be imported are listed under Import Reagents.
   - The associated negative control link(s) to be imported are listed under Import Negative Control.

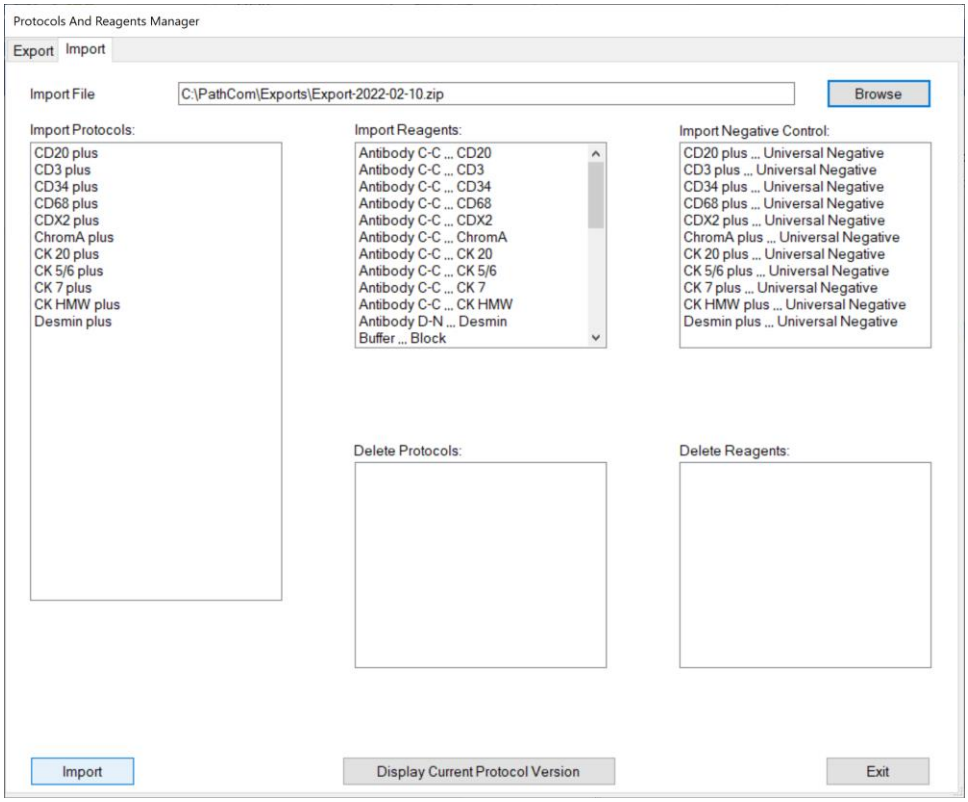


Figure 57

c. Click "**Import**" to import the contents of the export package.
   - A status bar will be displayed to show the progress of the import process.

   Note: Special protocols, templates, and reagents sharing the same name will be overwritten.

   Protocol customizations for standard protocols that share the same name will be overwritten. Standard protocols will be automatically refreshed at the end of the import process.
   - The system will display a confirmation message "Finished' when the process is completed.

d. Click "**Exit**" to close the utility.

Import Process

a. Standard protocol: The protocol is imported to the corresponding tab of the assigned Protocol Template.
   - If the protocol already exists on the system, it will be overwritten.
   - If the assigned Protocol Template's tab does not exist on the system, it will be created.
   - The saved customizations are updated with the imported protocol's customizations.
   - The protocol is refreshed with the newly saved customizations and the assigned Protocol Template.
   - The negative protocol is generated.
   - If the assigned Protocol Template does not exist on the system and was not imported together with the protocol, the protocol cannot be refreshed. The user will be prompted with an error and must create, import, or assign a new template.

b. Protocol template: The template is imported to the Template tab.
   - If the template already exists on the system, it will be overwritten.
   - If the assigned Protocol Type tab does not exist on the system, it will be created.
   - If the assigned Protocol Type is changed, all associated protocols will move to the new tab.
   - All protocols assigned to the template will be refreshed.

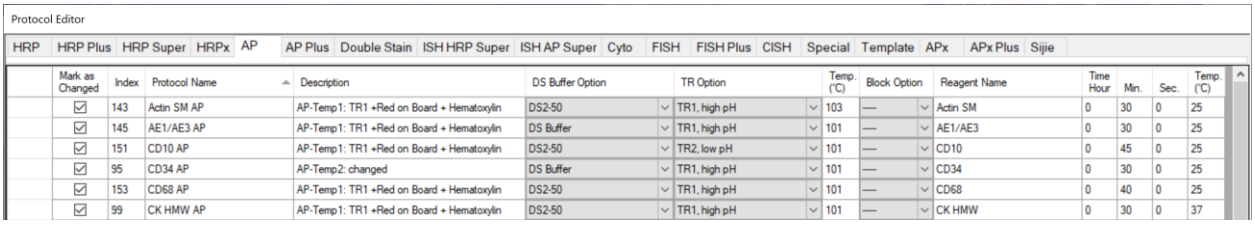
c. Special protocol: The special protocol is imported to the Special tab.
   - If the protocol already exists on the system, it will be overwritten.

5.3 Protocol Version Control

The protocol's Base Version may be used to keep track of different revisions of the protocols.
Note: This feature is optional.

The Base Version refers to the version of the manufacturer's Default Protocols and Default Reagents.

Protocols that are changed from the default are marked by a checked box under the column "Mark as Changed". This includes making modifications to protocol customizations, changing the template assignment, and adding new protocols.



Mark as Changed	Index	Protocol Name	Description	DS Buffer Option	TR Option	Temp. (°C)	Block Option	Reagent Name	Time Hour	Min.	Sec.	Temp. (°C)
☑	143	Actin SM AP	AP-Temp1: TR1 +Red on Board + Hematoxylin	DS2-50	TR1, high pH	103	---	Actin SM	0	30	0	25
☑	145	AE1/AE3 AP	AP-Temp1: TR1 +Red on Board + Hematoxylin	DS Buffer	TR1, high pH	101	---	AE1/AE3	0	30	0	25
☑	151	CD10 AP	AP-Temp1: TR1 +Red on Board + Hematoxylin	DS2-50	TR2, low pH	101	---	CD10	0	45	0	25
☑	95	CD34 AP	AP-Temp2: changed	DS Buffer	TR1, high pH	101	---	CD34	0	30	0	25
☑	153	CD68 AP	AP-Temp1: TR1 +Red on Board + Hematoxylin	DS2-50	TR1, high pH	101	---	CD68	0	40	0	25
☑	99	CK HMW AP	AP-Temp1: TR1 +Red on Board + Hematoxylin	DS2-50	TR1, high pH	101	---	CK HMW	0	30	0	37

Figure 58

The user may wish to change the manufacturer's Default Protocols and Default Reagents and update the protocol's Base Version to track a major protocol update.

Viewing the Protocol Version

To view the current protocols' Base Version, click "**Version**" in the Protocol Editor.
Alternately, click "**Display Current Protocol Version**" in the PR Manager.

The Base Version is listed above a sub-list of imported Export Packages, listed in order by the export date.

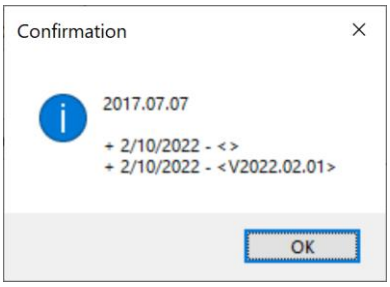


Figure 59

Changing the Protocol Version

To update the Base Version using an export package, select the Export Type: Base Version Update.

a. Check the box **Export Type: Base Version Update**

b. Click "**Set New Current Version and Default**"
   - Enter the new **Version** number.
   - Enter the new **Version Information**.
   - Click "**OK**" to change the protocol Base Version.

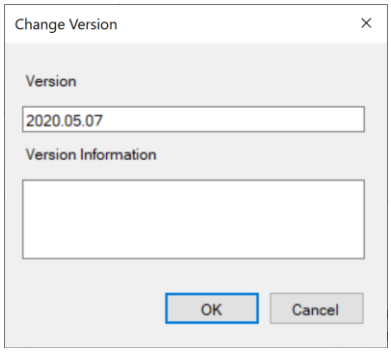


Figure 60

c. The (Exporter's) Current Protocol Version will be updated to reflect the new protocol Base Version, and a new set of Default Protocols and Default Reagents files will be automatically generated.

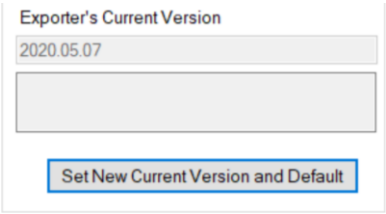


Figure 61

d. Proceed to create the Export Package. *Refer to Creating an Export Package.*
   - When the end-user imports the Base Version Update, the Current Protocol Version and the Default Protocols and Default Reagents will be automatically updated in addition to the imported protocols.

Section 6: Staining Area

6.1 Staining Area Map

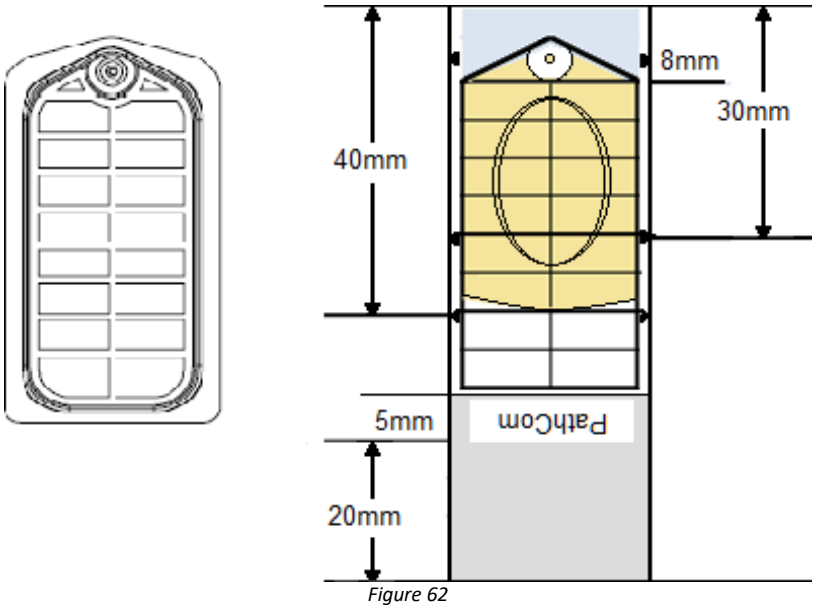


Figure 62

Estimated Staining Area based on Chamber Size

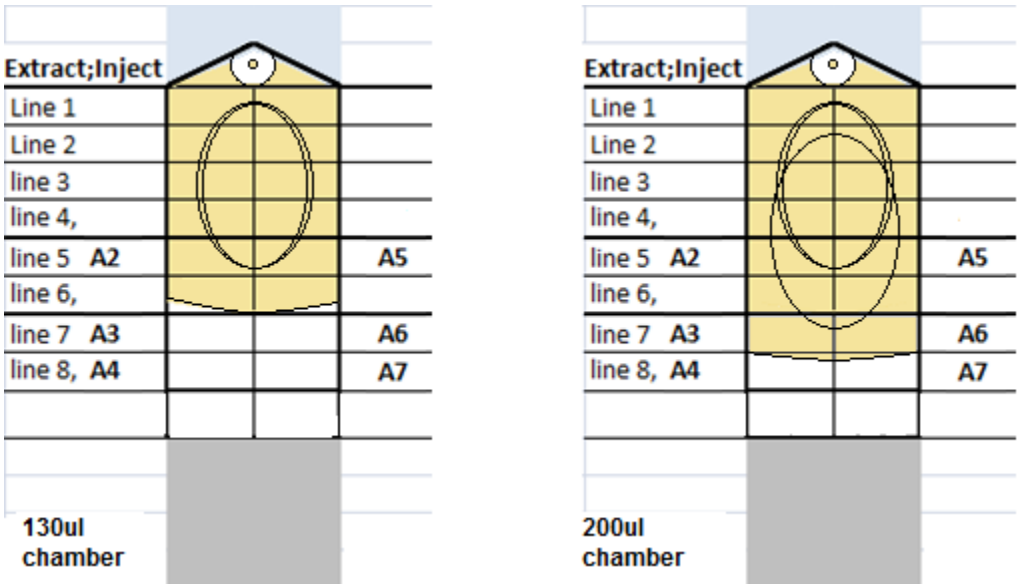


Figure 63

6.2 Slide Specifications

Slide Dimensions

The standard slide dimensions are 75mm x 25mm x 1.0mm.

a. The module's side rails and front clip must be adjusted to accommodate longer and wider slides.
b. The module's heater height must be adjusted to accommodate thicker/thinner slides.
c. The modules calibration angles must be adjusted to accommodate thicker/thinner slides.

Tissue Thickness

The recommended tissue thickness is 2-5 microns.

Tissue Placement

Mount tissues sections at position A and B to evaluate the staining area of a specific staining application.

1) Position A will verify uniformity near the injection site.
2) Position B will verify uniformity at the edge of the staining area, subject to evaporation.

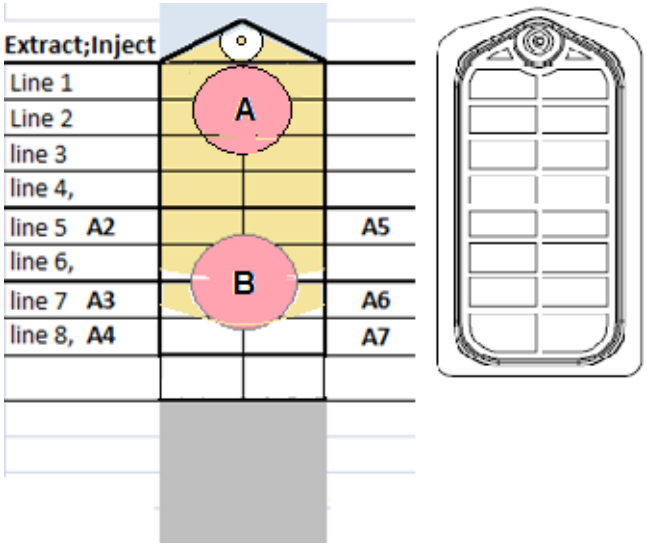


Figure 64

Section 7: Troubleshooting

7.1 Report an Issue

The user may report an issue to Technical Support for troubleshooting assistance.

Serial Number
Provide the instrument serial number for case tracking purposes.

System Software Version
Provide the system software to report errors/bugs.

Remote Access Information
Provide TeamViewer ID and Password to grant remote control.

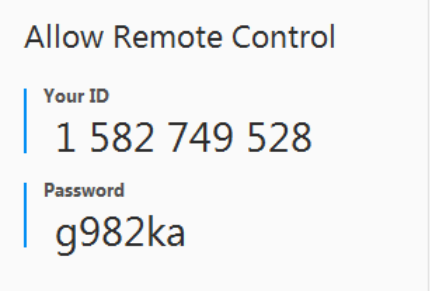


Figure 65

System Log Files
Provide the log files to assist in troubleshooting.
Sort the files by DateModified to provide the most recent logs.

C:\PathCom\PathComASGui\Logs
C:\PathCom\PathComAutostainer\Logs

System Data Files
Provide the data files to assist in reproducing reported issues.

C:\PathCom\PathComASGui\Data
C:\PathCom\PathComAutostainer\Data
C:\PathCom\PathComAutostainer\PositionCorrectionData

7.2 System Error and Failure Cases

Case 1: System Abort
All aborted slides will be highlighted in orange on the slide map.

The system may abort if it encounters a severe error while running the staining process, due to :
a. Loss of power due to power outage or power cable connection
b. Loss of communication due to USB cable connection
c. Pump overload due to a clog in the tubing lines/probes or syringe wear
d. Failure of the robotic arm due to wear or obstruction
e. Failure of the electronic boards or overloaded power supply

Click "**OK**" and initialize the system.
Unload slides. There will be reagent from the aborted process remaining on the slides.
Wipe and clean modules before starting a new staining process.

Case 2: System Frozen
The staining run will stop progressing. The software application may be Not Responding, locked or greyed out.
The run progress is visible in the Autostainer Server>Gantt tab.
Expand the Autostainer Server from the taskbar, if possible, to confirm this is the case.

The system may freeze if the software application crashes, due to:
a. Background PC processes consume too much CPU/Disk/Memory resources
b. Disk Defragmentation
c. Antivirus Scan
d. Windows Update
e. Windows Time Synchronization
f. Scheduled Task: Synchronize Language Settings (when Region/Language setting is changed from U.S./English)
g. Changing the taskbar display
h. Changing file/folder options in the Windows system
i. Log in/out of remote TeamViewer session

Click "**Exit**" (it may take a few minutes to error-out before closing the application).
Alternatively, input Ctrl+Alt+Del to open the Task Manager and kill the processes for PCStainer.exe and PathComAutostainer.exe

Log into the PCStainer application.
Unload slides. There will be reagent from the aborted process remaining on the slides.
Wipe and clean modules before starting a new staining process.

Case 3: System "Pushing"/Stalls
The run will stop progressing. One step of the staining process will fail to finish and continuously "push" the schedule, preventing other steps from starting.
The run progress is visible in the Autostainer Server>Gantt tab.
Expand the Autostainer Server from the taskbar, if possible, to confirm this is the case.

The system may "push"/stall due to:
a. Background PC processes consume too much CPU/Disk/Memory resources
b. Windows Time Synchronization
c. Scheduled Task: Synchronize Language Settings (when Region/Language setting is changed from U.S./English)

Click "**Exit**" (it may take a few minutes to error-out before closing the application).

Alternatively, open the Task Manager and kill the processes for PCStainer.exe and PathComAutostainer.exe

Log into the PCStainer application.
Unload slides. There will be reagent from the aborted process remaining on the slides.
Wipe and clean modules before starting a new staining process.

Case 4: System Error and Warnings

All slides potentially affected by the error will be highlighted in yellow on the slide map.

The system may encounter sporadic errors due to:

a. Insufficient reagent or liquid detection failure
b. Overheating; heater sensor failure
c. Low Temperature; insufficient cool down time or heater failure
d. Failure of the lid to reach extraction position in time
e. Failure to complete a step in the staining process in the allotted time
f. Loss of communication to one or more devices

Unload slides. Verify staining results.
Click System Utilities>Tools>Check Modules to verify module function, if applicable.
Check the volume remaining in reagent vials, if applicable.

Case 5: Module Malfunction

The slide will be highlighted in red on the slide map.
The system will skip this slide for the remainder of the staining process.

The module may malfunction due to:

a. Failure of the electronic board; liquid damage/short
b. Bad connection
c. Incorrect calibration or corrupted data

Unload slides. *It may or may not be possible to remove the slide from the module.*
Click System Utilities>Tools>Check Modules to verify module function.
The module cannot be operated until the cause of the malfunction is resolved.

Case 6: Waste Station Overflow

The system will sound an alarm and display a pop-up error on the screen.
The system will verify the liquid level in the waste station during probe rinse/wash at the end of each step.

The alarm may be triggered due to:

d. Overflow of waste bottles
e. Clog/obstruction in the waste tubing or waste station
f. Poor angle/drainage of the waste tubing
g. Failure of the liquid detection sensor

Empty the waste bottles and ensure the tubing is draining properly.

7.3 Staining Quality Issues

Case 1: Weak Staining

Note: Exceptions may occur. Some of the below factors may also cause weaker staining intensity. Test each condition individually to identify which conditions most significantly impacts staining intensity.

a. DS Buffer (System Fluid)->DS2
   - Exceptions: OCT-2, Chrom A, CD138 may get stronger staining with DS Buffer

b. Increasing TR temperature from 101C to 103C
   - Exceptions: CD20 may get better results at 99/98c

c. TR2, low pH->TR1, high pH retrieval solution
   - Exceptions: For a very few specific antibodies, may get stronger staining with TR2

d. TR1, high pH->TR4, high pH retrieval solution
   - For a few specific antibodies, such as ER and PR, may get stronger staining withTR4

e. 1-step detection-> 2-step detection
   - For some mouse antibodies, may get stronger staining with 2-step

f. 2-step detection->3-step detection

g. Single TR with 3-step detection->Double TR with 3-step detection

h. Replace DS/TR Option with special reagents
   - DS Enzyme or customer's own selection such as protease K, pronase
   - TR Enzyme or customer's own selection such as protease K, pronase
   - Combine Enzyme Retrieval + Heat-mediated Retrieval

i. Increase the antibody concentration

j. Change the antibody clone

k. Verify reagent quality

Case 2: Overstaining

a. Decrease the antibody concentration
   - Time from 30 to 15 minutes (minimum 10 minutes)

b. Decrease antibody incubation time

c. Decrease antibody incubation temperature
   - Range RT(25C) to 37C

d. Decrease TR conditions:
   - TR1->TR2
   - 101c->98/99c

Case 3: Negative Staining

a. Verify presence/absence of wax

b. Verify presence/absence of counterstaining

c. Verify presence/absence of tissue
   - Verify baking time and temperature
   - Verify slides, positively charged
   - Verify tissue fixation

d. If all slides are negative:
   - Verify common reagents; esp. storage of light-sensitive and temperature-sensitive reagents
   - Verify wash buffer
   - Verify chamber cleaning procedure
   - Verify robot and pump function

e. If only one slide of the same tissue is negative:
   - Verify slide/tissue is oriented face up
   - Verify sufficient reagent volume in the vial(s)
   - Verify chamber
   - Verify module

f. If only one module position is repeatedly negative/weak:
   - Verify calibration XYZ/agitation angles of the module
   - Perform module check
g. If only slides of one tissue are negative:
   - Verify tissue is mounted in the designated staining area
   - Verify tissue pretreatment
   - Use freshly cut tissue
   - Adjust protocol for that tissue type
h. If all/many slides of one antibody are negative:
   - Verify General Recommendations
   - Change to a different lot or vial of antibody
   - Change the tissue (2 or more other tissues and/or tissues from different sources that are verified positive to test the antibody)
   - Adjust protocol to enhance staining intensity conditions (set a common good antibody and tissue as the reference, such as CD3 on tonsil)

Case 4: Inconsistent Staining

a. Verify same/different tissues.
b. Verify the tissue pretreatment (fixation), very common issue.
c. Overall weak intensity, protocol not robust.
   - DS2 vs. DS Buffer
   - Primary antibody temperature: 25c vs 37C, 33C, or 45C
   - Increase TR temperature 101->103C
   - 1-step -> 2-step detection for some mouse antibodies
   - DS Buffer (System Buffer) ->DS2
d. Verify Ab clone
   - For example, Ki67 (SP6) is more consistent than MIB-1 clone.
e. Verify chamber condition; dirty or cracked chambers.
f. Verify module condition; module malfunction, wrong agitation position, angle calibration.
g. Verify reference positions; robot XYZ calibration
h. Verify tubing; tubing clogged or contaminated
i. Verify pumps; syringe wear or pump malfunction causes inaccurate aspiration/dispensation.
j. Verify wash buffer; replace with fresh buffer.
k. Verify common reagents; contamination, pro-longed use at RT.

Case 5: Other Staining Concerns

a. High background/hue on slides
   - Clean chambers
   - Clean tubing
   - Verify pump and robot function; poor extraction
   - Verify slide quality
   - Set Delay Start, to prevent stained slides from sitting in the system too long.
   - Replace or prepare fresh DAB
b. Debris accumulates around the chamber
   - Clean chambers
   - Verify pump and robot function; poor extraction
   - Set Delay Start, a small volume of reagent may flow to the chamber edge and dry.
   - Add wash step after HRP
   - Verify substrate/chromogen quality for precipitation and contamination
c. Gradient/reduced staining area
   - Verify the chamber
   - Verify the module calibration

- Verify evaporation rate of reagents/agitation angle and volume set in the protocol
- Verify TR temperature

d. Background/non-specific staining
   - Verify Ab clone or dilution
   - Verify blocking, H2O2 or protein block
   - Verify “effective” and set incubation times

e. Bubbles; small defined areas or circles with negative staining
   - Clean or replace chamber; surface uneven
   - Adjust protocol; increase agitation frequency

f. “Patchy” staining; areas of weaker staining, particularly towards center of tissue or on large tissues
   - Clean chambers
   - Adjust protocol; increase agitation frequency
   - Adjust protocol; enhance retrieval/pretreatment conditions
   - Verify reagent volume/concentration
   - Replace on-board DAB with pre-mixed DAB
   - Verify tissue fixation

g. Morphology; pathologists’ preference
   - *Note: May weaken staining intensity. Increase antibody concentration or enhance retrieval conditions to achieve a good balance.*
   - Verify tissue fixation (most important factor)
   - TR1->TR2, low pH
   - Reduce TR incubation time
   - Reduce TR temperature (not as significant)

h. DAB color; pathologists’ preference for “black/dark brown” DAB
   - Hematoxylin-> Hematoxlyn E (DAB Enhancer)

Appendix I: Recommended IHC Protocols

Abs Qualified on SS1					Protocol Special Requirements and Recommendation					
No.	**Name**	**Clone**	**Dilution**	**Ab Source**	**DS2**	**TR1**	**101C**	**Ab Time**	**Ab Temp**	**Polymer**
1	ACTH	Pab	1:100	CellMarque						
2	Actin, MS	HHF35	1:25	CellMarque						
3	Actin, MS	HHF35	RTU	LV			25C			
4	Actin, SM	1A4	1:100	CellMarque		TR2 prefer				
5	Actin, SM	1A4	RTU	LV		TR2	80C			
6	AR	AR441	RTU	Maxim						
7	ALK/p80	PAb	RTU	Maxim						
8	Bcl-2	EP36	1:100	Epitomics						
9	Bcl-2	124	1:100	CellMarque			103C	59m	25C	2-step
10	Bcl-2	100/D5	1:40	Klinipath			103C	59m	25C	2-step
11	Bcl-2	100/D5	RTU	LV	DS Buffer		103C	30m	25C	
12	Bcl-6	LN22	RTU	Maxim						
13	Bob-1	PAb	RTU	Maxim		TR2				
14	C3d	Pab	1:50	CellMarque		TR2 prefer				
15	CA125	EP48	1:100	Epitomics						
16	CA125	TA347	RTU	Maxim	DS buffer					
17	CA15-3	DF3	RTU	Maxim						
18	CA19-9	TA888	RTU	Maxim						
19	Carbonic Antydrase IX	PAb	RTU	Maxim						
20	Calcitonin	EP92	1:100	Epitomics						
21	Calcitonin	Pab	1:200	CellMarque			103C			
22	Calponin	CALP	RTU	Maxim						
23	Calponin-1	EP63	1:100	Epitomics						
24	Calponin-1	EP798Y	1:50	CellMarque						
25	Calretinin	Pab	1:100	CellMarque						
26	Calretinin	Pab	RTU	LV		TR2	85C			
27	Calretinin	SP13	RTU	Maxim				59m	25C	
28	Catenin beta	EP35	1:100	Epitomics						
29	Catenin beta	Pab	1:100	CellMarque						
30	CD1a	O10	RTU	Maxim						
31	CD2	AB75	RTU	Maxim						
32	CD3	EP41	1:100	Epitomics						
33	CD3	SP7	1:450	Klinipath						
34	CD3	PS1	RTU	Leica						
35	CD4	SP35	1:40	Klinipath						
36	CD4	1F6	RTU	Leica						
37	CD5	EP77	1:100	Epitomics						
38	CD5	SP19	1:50	Dako						

39	CD5	4C7	RTU	CellMarque						
40	CD7	272	RTU	Maxim						
41	CD8	SP16	1:100	Spring						
42	CD8	C8/144B	1:25	CellMarque						
43	CD8	1A5	RTU	Leica						
44	CD10	56C6	RTU	Leica				59m	25c	
45	CD10	CD10.1	1:50	Klinipath			103C	59m	25c	
46	CD123		RTU	Maxim						
47	CD138	MI15	RTU	Maxim	BS Buffer			45m	33c	
48	CD15	MMA	1:25	CellMarque						
49	CD15	MMA	RTU	Maxim				45m	33c	
50	CD20	L26	1:100	Dako	DS buffer	TR2 prefer	98C			
51	CD207 (Langerin)	12D6	RTU	Maxim						
52	CD21	EP64	1:200	Epitomics						
53	CD21	EP3093	1:50	CellMarque						
54	CD23	EP75	1:300	Epitomics			103C			
55	CD23	SP23	1:100	Dako			103C			
56	CD235alpha	JC159	RTU	Maxim						
57	CD30	Ber-H2	1:50	CellMarque						
58	CD30	Ber-H2	RTU	Maxim				45m	33c	
59	CD31	EP78	1:200	Epitomics						
60	CD31	JC70	1:50	CellMarque		TR1				
61	CD34	EP88	1:100	Epitomics						
62	CD35/CR1	KuN241	RTU	Maxim						
63	CD43	MT1	1:50	CellMarque						
64	CD44	MRQ-13	1:100	CellMarque		TR2 prefer				
65	CD44v6	2F10	RTU	Maxim						
66	CD45/LCA	LCA	1:100	CellMarque					25C	
67	CD45/LCA	EP68	1:200	Epitomics					25C	
68	CD45RO	UCHL-1	1:50	CellMarque		TR2 prefer				
69	CD45RO	UCHL-1	RTU	Maxim			37c	45m	33c	
70	CD56	MRQ-42	1:100	CellMarque			100C			
71	CD56		RTU	Maxim	DS buffer		103c	50m	30c	
72	CD57	NK1	1:100	CellMarque						
73	CD61	2f2	1:25	CellMarque						
74	CD68	Kp-1	RTU	LV			103C			
75	CD68	514H12	RTU	Leica						
76	CD79a	EP82	1:400	Epitomics						
77	CD79a	SP18	1:200	Spring						
78	CD79a	JCB117	1:200	CellMarque						
79	CD79a	HM47/A 9	RTU	Maxim			103C			
80	CD99	EP8	1:100	Epitomics						

81	CD99	EP8	RTU	LV		TR2	95C			
82	CD99	EPR3097Y	1:100	CellMarque						
83	CD117	YR145	1:25	CellMarque						
84	CD117	2.00E+04	RTU	Maxim						
85	CD117	EP10	RTU	LV		TR2				
86	CD163	MRQ-26	1:10	CellMarque						
87	CDX2	EP25	1:100	Epitomics						
88	CDX2	DAK-CDX2	RTU	Dako						
89	CEA	CEA31	1:100	CellMarque						
90	c-erbB2	EP3	RTU	Epitomics	DS buffer	TR2	95C			
91	c-erbB2	EP36	1:50	Epitomics	DS buffer		100C			
92	c-erbB2	SP3	1:50	Spring	DS buffer		100C			
93	c-erbB2	PAb	RTU	Maxim	DS buffer		100C			
94	c-fos	PAb	RTU	Maxim						
95	Chromogranin A	EP38	1:100	Epitomics						
96	Chromogranin A	LK2H10	1:100	CellMarque						
97	Collagen IV	PHM-12	RTU	Maxim						
98	COX2	SP	1:100	Spring						
99	Cyclin D1	EP12	1:100	Epitomics						
100	Cyclin D1	SP4	1:25	Klinipath						
101	Cyclin D1	SP4	RTU	Maxim	DS Buffer		103c	45m	33c	
102	Cyclin E	HE12	RTU	Maxim						
103	Cytokeratin 5	EP24	1:200	Epitomics						
104	Cytokeratin 5	SP27	1:100	Spring						
105	Cytokeratin 5	XM26	RTU	Leica						
106	Cytokeratin 7	EP16	1:200	Epitomics						
107	Cytokeratin 7	OV-TL 12/30	1:150	Klinipath						
108	Cytokeratin 7	OV-TL 12/30	RTU	Maxim			99c			
109	Cytokeratin 8	35betaH11	1:50	CellMarque						
110	Cytokeratin 14	EP61	1:100	Epitomics						
111	Cytokeratin 14	SP53	1:100	Spring						
112	Cytokeratin 17	EP98	1:100	Epitomics						
113	Cytokeratin 17	K217.E3	1:25	CellMarque						
114	Cytokeratin	B22.1&B	1:100	CellMarque						

	8/18	23.1								
115	Cytokeratin 8/18	CAM 5.2	1:100	Klinipath						
116	Cytokeratin 5/6/8/18	5D3 and LP34	RTU	Leica						
117	Cytokeratin 19	EP72	1:200	Epitomics						
118	Cytokeratin 19	Ks19.1	RTU	LV			90C			
119	Cytokeratin 20	EP23	1:100	Epitomics						
120	Cytokeratin 20	Ks20.8	RTU	Maxim			99c			
121	Cytokeratin, HMW	34betaE12	1:100	CellMarque						
122	Cytokeratin, HMW	34betaE12	RTU	LV	DS Buffer	TR2	95C			
123	Cytokeratin, HMW	AE3	1:100	CellMarque						
124	Cytokeratin, LMW	AE1	1:100	CellMarque						
125	Cytokeratin, pan	AE1/AE3	RTU	Leica						
126	Cytokeratin, pan	AE1/AE3	RTU	LV	DS buffer	TR2	90C			
127	D2-40	D2-40	RTU	Maxim	DS buffer					
128	EBV	CS1-4	RTU	Maxim						
129	E-cadherin	EP6	1:200	Epitomics						
130	E-cadherin	NCH-38	1:25	Klinipath						
131	EGFR	EP22	1:100	Epitomics						
132	EGFR	H11	RTU	Maxim		TR enzyme	35C			
133	ER	SP1	RTU	LV		TR2	95C			
134	ER	EP1	1:50	Epitomics						
135	ER	1D5	1:50	Klinipath				59m	25C	
136	ERCC1	8F1	RTU	Maxim						
137	ESA	Ber-EP4	1:50	CellMarque		TR2				
138	Factor XIIIa	EP3372	1:100	CellMarque						
139	Fli-1	MRQ-1	1:25	CellMarque						2-step
140	Fli-1	G146-222	RTU	Maxim						
141	FSH	Pab	1:25	CellMarque						
142	Fas (CD95)	PAb	RTU	Maxim						
143	Fibronectin	PAb	RTU	Maxim						
144	Galectin-3	MM(9C4)	RTU	Maxim	DS buffer					
145	Gastrin	PAb	RTU	Maxim						
146	GCDFP-15	23A3	RTU	Maxim						
147	GFAP	EP13	1:100	Epitomics						

148	GFAP	GA5	1:50	CellMarque						
149	GH	Pab	1:100	CellMarque			103C			
150	Glypican-3	1G12	1:50	CellMarque			103C			
151	GST-pai		RTU	Maxim					25c	
152	HBcAg	PAb	RTU	Maxim			25C			
153	HBsAg	3.00E+07	RTU	Maxim			37c	50m	33c	
154	HCG alpha	PAb	RTU	Maxim						
155	HCG beta	ZSH17	RTU	Maxim						
156	Hepatocyte Specific Antigen	OCH1E5	1:100	CellMarque		TR2 prefer				
157	HSP27	G3.1	RTU	Maxim						
158	IgG4	HP6025	RTU	Maxim						
159	INI1	MRQ-27	1:50	CellMarque						2-step
160	Inhibin alpha	R1	RTU	Maxim	DS enzyme			45m	33c	
161	Insulin	INS04+INS05	RTU	Maxim						
162	Kappa Light Chain	L1C1	1:100	CellMarque		TR2 prefer	103C			
163	Ki-67	K2	1:200	Klinipath						
164	Ki-67	SP6	1:200	Spring						
165	Ki-67	EP5	1:100	Epitomics						
166	Lambda	Lamb14	1:100	CellMarque		TR2 prefer				
167	Laminin	LAM-89	RTU	Maxim						
168	LRP	1032	RTU	Maxim						
169	Lysozyme	PAb	RTU	Maxim						
170	Macrophage	HAM-56	1:25	CellMarque		TR2 prefer				
171	Mammaglobulin	304-1A5	RTU	Maxim						
172	MART-1	EP43	1:100	Epitomics						
173	MART-1		1:100	Klinipath						
174	MART-1	M2-7C10	1:100	CellMarque						
175	MBP	PAb	RTU	Maxim						
176	MDM2	PAb	1:50	Santa Cruz		TR2?				
177	Melanoma	HMB-45	1:100	CellMarque						
178	MITF	C5/D5	1:50	CellMarque						
179	MLH1	G168-728	1:25	CellMarque						
180	MLH1	G168-15	RTU	Maxim						
181	Moc31	MOC-31	RTU	Maxim						
182	MRP1	33A6	RTU	Maxim						
183	MRP3	DTX1	RTU	Maxim						
184	MSH2	FE11	RTU	LV			103C			
185	MSH6	BC/44	1:50	Klinipath						
186	MSH6	44	RTU	Maxim			103C			
187	Mucin 2	M53	RTU	Maxim						

188	MUM1	MRQ-43	1:50	CellMarque						
189	MUM1	MUM1p	RTU	Maxim						
190	MUM1	RbMAb	RTU	LV		TR2	90C			
191	Myeloid	MAC387	RTU	Maxim						
192	Myeloperoxidase	Pab	1:50	CellMarque						
193	Myogenin	F5D	RTU	Maxim						
194	Myoglobin	EP87	1:100	Epitomics						
195	Myoglobin	Pab	1:50	CellMarque						
196	Myosin	SMMS-1	RTU	Maxim						
197	Nestin	10C2	RTU	Maxim						
198	Neurofilament	2F11	1:100	CellMarque						
199	NSE	E27	1:50	CellMarque						
200	nm23	PAb	RTU	Maxim						
201	Nuclear Protein	A6O	RTU	Maxim						
202	Oct3/4	C-10	RTU	Maxim						
203	p120 catenin	98/pp20	RTU	Maxim						
204	p16	PAb	RTU	Maxim						
205	p21	DCS-60.2	1:50	CellMarque						
206	p27	SX53G8	1:100	CellMarque						
207	p53	EP9	1:100	Epitomics						
208	p53	DO7	1:100	CellMarque						
209	p53	SP5	1:100	Spring						
210	p57	Kp10	1:100	CellMarque						
211	p63	4A4	1:100	Klinipath						
212	P504S	13H4	RTU	Maxim			103C	45m	33c	
213	Papilloma Virus	PAb	RTU	Maxim						
214	Pax-5	PAb	RTU	Maxim			103C	45m	33c	
215	Pax-8	Pab	1:25	CellMarque				59m	25C	
216	Pax-8	BC12	RTU	LV			103C			
217	PGP9.5	PAb	RTU	Maxim	DS buffer			50m	30c	
218	PLAP	NB10	1:100	CellMarque						
219	PLAP	SP15	1:50	Spring						
220	Progeterone Receptor	EP2	1:100	Epitomics						
221	Progeterone Receptor	SP2	1:50	Spring				45m	33c	
222	Prolactin	Pab	1:100	CellMarque						
223	RRM1	PAb	RTU	Maxim						
224	pS2 protein	BC04	RTU	Maxim						
225	PSA	ER-PR8	1:25	CellMarque						
226	PSA	PSA28/A4	RTU	Leica						
227	PSA	EP109	RTU	Epitomics		TR2	90C			
228	PSAP	PASE/4LJ	RTU	Maxim						

229	PSMA	SP29	1:100	Spring						
230	Rb Gene protein	PAb	RTU	Maxim	DS enzyme			45m	33c	
231	Renal Cell Carcinoma	PN-15	1:25	CellMarque		TR2 prefer				
232	S100	4C4.9	1:100	CellMarque						
233	S100	4C4.9	RTU	LV		TR2	70C			
234	S100	PAb	RTU	Leica						
235	Surfactant Protein A	PE10	RTU	Maxim						
236	Surfactant Protein B	SBP01	RTU	Maxim						
237	Survivin	PAb	RTU	Maxim						
238	Synaptophysin	SP11	1:100	Spring						
239	TAG-72	B72.3	1:100	CellMarque		TR2				
240	TdT	Pab	1:100	CellMarque			100C			
241	TdT	SEN28	RTU	Maxim				45m	33c	
242	TGF beta1		RTU	Maxim						
243	Thyroglobulin	TGB04+TGB05	RTU	Maxim						
244	Thyroglobulin	2H11+6E1	1:100	CellMarque		TR2				
245	Tia-1	Tia-1	RTU	Maxim						
246	Topo II		RTU	Maxim			103c	50m	30c	
247	TPO	AC25	RTU	Maxim						
248	Thymidine Synthase	TS106	RTU	Maxim			103C			
249	TSH	Pab	1:50	CellMarque						
250	TTF-1	8G7G3/1	1:100	CellMarque						
251	TTF-1	SPT24	RTU	Leica						
252	beta Tubulin III	TUJ1	RTU	Maxim						
253	Villin	CWWB1	RTU	Maxim						
254	Tyrosinase	T311	1:100	CellMarque		TR2 prefer				
255	VEGF	PAb	RTU	Maxim						
256	Vimentin	EP21	1:200	Epitomics						
257	Vimentin	SP20	1:200	Spring						
258	Vimentin	V9	RTU	LV		TR2	90C			
259	WT1	EP122	1:100	Epitomics						
260	WT1	6F-H2	1:50	CellMarque						
261	WT1	WT49	RTU	Maxim			103C	59m	25C	
262	ZAP-70	EP52	1:100	Epitomics						
263	ZAP 70	2F3.2	1:100	CellMarque		TR2 prefer				