NeoPATH Pro-Tect HRP Detection Kit

Micro-polymer detection
901-10031-050925

Available Product Formats	
Catalog Number	**Volume**
NPRI10031T135	135 tests

Intended Use:
For *in vitro* Diagnostic Use
The NeoPATH Pro-Tect HRP Detection Kit is intended for use in automated Immunohistochemistry (IHC) staining protocols using a horseradish peroxidase (HRP) polymer two/three-step application method. This micro-polymer detection kit is designed for the detection of mouse IgG, and/or rabbit IgG primary antibodies bound to target antigens in the formalin-fixed, paraffin-embedded (FFPE) tissues during the IHC staining process. The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
The NeoPATH Pro-Tect HRP Detection Kit is designed using a two-step or three-step method for detecting mouse and/or rabbit primary antibodies to form an antibody-enzyme complex. This complex is then visualized using an appropriate substrate/chromogen. In the two-step method (rabbit), the universal linker is applied, then an additional enzyme-linked polymer labeled reagent is sequentially applied. In the three-step method (mouse), the mouse probe is applied, followed by the universal linker, then an additional enzyme-linked polymer labeled reagent is sequentially applied.
NeoPATH Pro-Tect HRP Detection Kit is provided ready-to-use and is intended to be applied as defined by the staining protocols on the NeoPATH Pro automated stainer.

Principle of Procedure:
This micro-polymer detection kit may be used in immunohistochemistry testing of formalin-fixed, paraffin-embedded tissue sections. In general, immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody (optional link antibody/probe), an enzyme complex and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained, and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Materials and Methods:

Reagents Provided:

Kit Catalog No.	Component Catalog No.	Component Description	Quantity x Volume
NPRI10031T135	NPRI10032T135	NeoPATH Pro-Tect Mouse Probe	1 x 45 mL
	NPRI10033T135	NeoPATH Pro-Tect Linker	1 x 45 mL
	NPRI10034T135	NeoPATH Pro-Tect HRP Polymer	1 x 45 mL
	NPRI10035T135	NeoPATH Pro DAB Buffer	1 x 41 mL
	NPRI10036T135	NeoPATH Pro DAB Chromogen	1 x 8 mL
	NPRI10037T135	NeoPATH Pro Peroxidase	1 x 45 mL

Reconstitution, Mixing, Dilution, Titration:
The NeoPATH Pro-Tect HRP Detection Kit is optimized and ready to use with Biocare IHC antibodies and ancillary reagents. No reconstitution, mixing, dilution or titration is required.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity:
Mouse and Rabbit IgG heavy and light chains

Supplied As:
NeoPATH Pro-Tect Mouse Probe-NPRI10032
Buffered saline solution, pH 7.2-7.4, containing a protein carrier and less than 0.1% sodium azide preservative. See Safety Data Sheet for additional details.

NeoPATH Pro-Tect Linker- NPRI10033
Buffered saline solution, pH 7.2-7.4, containing a protein carrier and less than 0.01% ProClin 300 and/or less than 0.5% ProClin 950 as a preservative. See Safety Data Sheet for additional details.

NeoPATH Pro-Tect HRP Polymer-NPRI10034
Buffered saline solution, pH 7.6-7.8, containing a protein carrier and less than 0.01% ProClin 300 and/or less than 0.5% ProClin 950 as a preservative. See Safety Data Sheet for additional details.

NeoPATH Pro DAB Buffer – NPRI10035
Buffered solution contains 3% Hydrogen Peroxide solution. See Safety Data Sheet for additional details.

NeoPATH Pro DAB Chromogen – NPRI10036
DAB solution. See Safety Data Sheet for additional details.

NeoPATH Pro Peroxidase – NPRI10037
Buffered saline solution, contains 1% hydrogen peroxide, pH 6.5 – 6.7, and less than 0.1% Sodium Azide preservative. See Safety Data Sheet for additional details.

Materials and Reagents Needed but Not Provided:
Microscope slides, positively charged
Positive and negative tissue controls
Desert Chamber* or similar Drying oven (optional)
Xylene or xylene substitute
Ethanol or reagent alcohol
Decloaking Chamber* or similar pressure cooker (optional)
Deionized or distilled water
Wash buffer*
Pretreatment reagents* (optional)
Enzyme digestion* (optional)
Protein block* (optional)
Primary antibody*
Negative control reagents*
Hematoxylin* (counterstain)
Bluing reagent*
Mounting medium*
Coverglass
Light Microscope (40-400X magnification)
Automated Slide Staining Platform

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* Biocare Medical Products: Refer to the Biocare Medical website located at http://biocare.net for information regarding catalog numbers and ordering. Certain reagents listed above are based on specific applications and detection system used.

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the vial label when stored under these conditions. Do not use after expiration date. Storage under any condition other than those specified must be verified. The kit reagent(s) are ready-to-use and should not be diluted. The stability of user diluted reagents has not been established by Biocare.

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed, which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Biocare's Technical Support at 1-800-542-2002 or via the technical support information provided on biocare.net.

Specimen Preparation:
Tissues fixed in formalin are suitable for use prior to paraffin embedding. Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades.[1,2]

Properly fixed and embedded tissues expressing the specified antigen target should be stored in a cool place. The Clinical Laboratory Improvement Act (CLIA) of 1988 requires in 42 CFR §493.1259(b) that "The laboratory must retain stained slides at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination."[3]

Treatment of Tissues Prior to Staining:
Perform Heat Induced Epitope Retrieval (HIER) per recommended protocol below. The routine use of HIER prior to IHC has been shown to minimize inconsistency and standardize staining. [4,5]

Warning and Precautions:
1. DAB is known to be a suspected carcinogen.
2. Do not expose DAB components to strong light or direct sunlight.
3. DAB may cause sensitization of skin. Avoid contact with skin and eyes.
4. Wear gloves and protective clothing and take reasonable precautions when handling DAB is classified as a danger and may cause cancer and is suspected of causing genetic defects.
5. Kit reagent(s) contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976)[6]
6. Kit reagents contain less than 0.05% ProClin 300 and/or less than 1% ProClin 950. Wear gloves and protective clothing and take reasonable precautions when handling as ProClin is classified as an irritant and may cause skin contact sensitization. Avoid contact with eyes, skin, and mucous membranes.
7. Handle materials of human or animal origin as potentially biohazardous and dispose of such materials with proper precautions. In the event of exposure, follow the health directives of the responsible authorities where used.[7,8]
8. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water.[9]
9. Microbial contamination of reagents may result in an increase in nonspecific staining.
10. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
11. Do not use reagent after the expiration date printed on the vial.
12. To prevent evaporation and ensure maximum test capacity, promptly cap and remove reagents from automated instruments after each run. Leaving reagents exposed can reduce their effectiveness and the number of tests they can provide. Always store reagents as directed to maintain their integrity.
13. The micro-polymer detection kit reagent(s) are optimized and ready to use with Biocare antibodies and ancillary reagents. Refer to the primary antibody and other ancillary reagent instructions for use for recommended protocols and conditions for use.
14. Dispose of all used reagents and any other contaminated disposable materials following procedures for infectious or potentially infectious waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to treat and dispose of it (or have them treated and disposed of) in accordance with any applicable regulations.
15. Follow local disposal regulations for your location along with recommendations in the Safety Data Sheet to determine the safe disposal of this product
16. The SDS is available upon request and is located at http://biocare.net.
17. Report any serious incidents related to this device by contacting the local Biocare representative and the applicable competent authority of the Member State or country where the user is located.

This NeoPATH Pro-Tect HRP Detection Kit contains components classified as indicated in the table below in accordance with Regulation (EC) No. 1272/2008.

Hazard	Code	Hazard Statement
	H317	May cause an allergic skin reaction.
	H341 H350	Suspected of causing genetic defects. May cause cancer.
N/A	H402 H412	Harmful to aquatic life. Harmful to aquatic life with long lasting effects.

Instructions for Use:
The micro-polymer detection kit reagent(s) are optimized and ready to use with Biocare antibodies and ancillary reagents. Refer to the primary antibody and other ancillary reagent instructions for use for recommended protocols and conditions for use. Incubation times and temperatures will vary depending on the specific antibody protocol followed.

When using an automated staining instrument, consult the specific instrument operator manual and instructions for use for operating parameters.

NeoPATH Pro-Tect HRP Detection Kit is provided in vials ready for use on the NeoPATH Pro Automated Slide Stainer. Uncap the vial and place in the NeoPATH Pro reagent tray. The NeoPATH Pro Atomated Slide Stainer will apply reagent as required in the selected protocol.

Quality Control:

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Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011[10]

Positive Tissue Control:
External positive control materials should be fresh specimens fixed, processed, and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are indicative of correctly prepared tissues and proper staining techniques. One positive external tissue control for each set of test conditions should be included in each staining run.

The tissues used for the external positive control materials should be selected from patient specimens with well-characterized low levels of the positive target activity that gives weak positive staining. The low level of positivity for external positive controls is designed to ensure detection of subtle changes in the primary antibody sensitivity from instability or problems with the IHC methodology. Commercially available tissue control slides or specimens processed differently from the patient sample(s) validate reagent performance only and do not verify tissue preparation.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, rather than as an aid in formulating a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control:
Use a negative tissue control fixed, processed, and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of the IHC primary antibody for demonstration of the target antigen, and to provide an indication of specific background staining (false positive staining). Also, the variety of different cell types present in most tissue sections can be used by the laboratorian as internal negative control sites to verify the IHC's performance specifications. The types and sources of specimens that may be used for negative tissue controls are listed within the respective primary antibody data sheet Performance Characteristics section.

If specific staining (false positive staining) occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Nonspecific Negative Reagent Control:
Use a nonspecific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. Ideally, a negative reagent control contains an antibody produced and prepared (i.e. diluted to same concentration using same diluent) for use in the same way as the primary antibody but exhibits no specific reactivity with human tissues in the same matrix/solution as the Biocare antibody. Diluent alone may be used as a less desirable alternative to the previously described negative reagent controls. The incubation period for the negative reagent control should correspond to that of the primary antibody.

When panels of several antibodies are used on serial sections, the negative staining areas of one slide may serve as a negative/nonspecific binding background control for other antibodies. To differentiate endogenous enzyme activity or nonspecific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate-chromogen or enzyme complexes (PAP, avidin-biotin, streptavidin) and substrate-chromogen, respectively.

Assay Verification:
Prior to initial use of an antibody or staining system in a diagnostic procedure, the user should verify the antibody's specificity by testing it on a series of in-house tissues with known immunohistochemical performance characteristics representing known positive and negative tissues. Refer to the quality control procedures previously outlined in this section of the product insert and to the quality control recommendations of the CAP Certification Program[11] for Immunohistochemistry and/or the NCCLS IHC guideline[12]. These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters.

Troubleshooting:
Follow the antibody specific protocol recommendations according to the data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

Interpretation of Staining:
A primary antibody works in conjunction with ancillary reagents to produce a colored reaction at the antigen sites localized by the primary antibody. Detection ancillary reagents assist with detecting the primary antibody binding in conjunction with a substrate-chromogen or enzyme complexes in the antibody-antigen specific staining reaction. Prior to interpretation of patient results, the staining of controls must be evaluated by a qualified pathologist. Negative controls are evaluated and compared to stained slides to ensure any staining observed is not a result of nonspecific interactions.

Positive Tissue Control:
The positive tissue control stained with indicated antibody should be examined first to ascertain that all reagents are functioning properly. The appropriate staining of target cells (as indicated above) is indicative of positive reactivity. If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid.

The color of the reaction product may vary depending on substrate chromogens used. Refer to substrate package inserts for expected color reactions. Further, metachromasia may be observed in variations of the method of staining.[13]

When a counterstain is used, depending on the incubation length and potency of the counterstain used, counterstaining will result in a coloration of the cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.

Negative Tissue Control:
The negative tissue control should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells/cellular components. If specific staining (false positive staining) occurs in the negative external tissue control, results with the patient specimen should be considered invalid.

Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

Patient Tissue:
Examine patient specimens stained with indicated antibody last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

Refer to Summary and Explanation, Limitations, and Performance Characteristics within the respective primary antibody data sheet for antibody immunoreactivity.

Limitations:

General Limitations:

1. For *in vitro* diagnostic (IVD) Use
2. This product is for professional use only: Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
3. For use by physician prescription only. (Rx Only)
4. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.[14]
5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology, and other histopathological criteria. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents, and methods to interpret all the steps used to prepare and interpret the final IHC preparation.
7. The optimum protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, antibody dilution, tissue section thickness and detection kit used. Refer to the primary antibody and other ancillary reagent instructions for use for recommended protocols and conditions for use. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.
8. This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry.
9. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[15]
10. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues.[16] Contact Biocare's Technical Support at 1-800-542-2002, or via the technical support information provided on biocare.net, with documented unexpected reaction(s).
11. Normal/nonimmune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.
12. False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudo peroxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g., liver, breast, brain, kidney) depending on the type of immunostain used.[14]
13. A negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue examined.

Product Specific Limitations:
No additional product specific limitations noted.

Performance Characteristics:
Analytical Performance:

NeoPATH Pro-Tect HRP Detection Kit is designed for the detection of mouse IgG, and/or rabbit IgG primary antibodies bound to target antigens in the formalin-fixed, paraffin-embedded (FFPE) tissues during the IHC staining process. As a standalone reagent, this product cannot be tested for specificity or sensitivity.

Multiple Biocare antibodies have been developed with the NeoPATH Pro-Tect HRP Detection Kit in IHC applications. As part of the testing for those assays, the following performance characteristics were demonstrated for the NeoPATH Pro-Tect HRP Detection Kit.

Staining was performed using protocols provided in the antibody specific instructions for use or as specified. Sensitivity and specificity of staining was evaluated across a range of normal and neoplastic tissue types evaluated during development of primary antibodies.

All studies met their acceptance criteria.

Troubleshooting:

1. No staining of any slides – Check to determine appropriate positive control tissue, antibody, and detection products have been used. Check for incomplete or improper wax removal or pretreatment.
2. Weak staining of all slides – Check to determine appropriate positive control tissue, antibody, and detection products have been used.
3. Excessive background of all slides – There may be high levels of endogenous biotin (if using biotin-based detection products), endogenous HRP activity converting chromogen to colored end product (use peroxidase block), or excess non-specific protein interaction (use a protein block, such as serum- or casein-based blocking solution).
4. Tissue sections wash off slides during incubation – Check slides to ensure they are positively charged.
5. Specific staining too dark – Check protocol to determine if proper antibody titer was applied to slide, as well as proper incubation times for all reagents. Additionally, ensure the protocol has enough washing steps to remove excess reagents after incubation steps are completed.

References:

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