Rat-on-Mouse AP-Polymer

Micro-polymer detection
902-RT518-032923

BIOCARE MEDICAL

Available Product Formats	
Catalog Number	Volume
RT518G	6.0 mL
RT518H	25 mL

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

Background Information:
Rat primary antibodies on mouse tissues are advantageous because rat secondary detection systems can be used with minimum cross-reactivity to endogenous mouse IgG. Biocare Medical has developed a Rat-on-Mouse alkaline phosphatase (AP) polymer detection system with minimal cross-reactivity to mouse tissues. It can be used with formalin-fixed paraffin-embedded tissues, floating sections, frozen sections, and cell culture preparations. The Rat-on-Mouse AP-Polymer is comprised of two reagents: a Rat Probe that binds to the rat primary antibody and a Rat-on-Mouse AP-Polymer that conjugates to the probe.

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

Supplied As:

Reagents Provided:

Kit Catalog No.	Component Catalog No.	Component Description	Quantity x Volume
RT518G	RTP629G	Rat Probe	1 x 6 mL
	RTAP631G	Rat-on-Mouse AP-Polymer	1 x 6 mL
RT518H	RTP629H	Rat Probe	1 x 25 mL
	RTAP631H	Rat-on-Mouse AP-Polymer	1 x 25 mL

* Refer to the Biocare Medical website located at http://biocare.net for information regarding catalog numbers and ordering.

Supplied As:
Rat Probe – RTP629
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.

Rat-on-Mouse AP-Polymer – RTAP631
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.

Reconstitution, Dilution and Mixing:
The Rat-on-Mouse HRP-Polymer reagent(s) are optimized and ready to use with Biocare antibodies and ancillary reagents. No reconstitution, mixing, dilution, or titration is required.

Species Reactivity:
Rat IgG with minimal cross-reactivity on mouse tissues.

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 reagent has not been established by Biocare.

Staining Protocol Recommendations:
Below are programming and protocol recommendations to assist the user when staining manually and/or using one of Biocare's Automated Staining Platforms for research applications. The user is responsible for further optimizations of the protocol.

1. Deparaffinize slides in Slide Brite or xylene.
2. Hydrate slides in a series of graded alcohols to water.
3. Optional: Post fix tissue in 10% formalin for 30 minutes. Wash in DI water. (See Technical Note #1)
4. Optional: Immerse slides in Peroxidazed 1 blocking reagent for 3-5 minutes. Wash in DI water. (See Technical Note #2)
5. Optional: Place slides in 1X Rodent Decloaker and heat using Biocare's Decloaking Chamber. (See Technical Note #3)
Heating times can be used as follows:
- 80˚C for 30-120 minutes or overnight for 12-18 hours
- 95˚C for 30-60 minutes
- 120-125˚C for 30 seconds or 5 minutes at high temperature
Remove slides and wash in DI water.
6. **Digestion Technique (Optional):**
- Place slides in Rodent Decloaker at 80˚C for 15-30 minutes. Wash in DI water. A post digestion can be performed using pepsin at room temperature (RT) for 1-5 minutes. Wash in TBS wash buffer.
- If Rodent Decloaker is not used, apply pepsin at RT for 10-15 minutes. Wash in DI water.
- If using Rodent Decloaker at 95ºC or 125˚C, a post-digestion can be performed using pepsin at RT for 30-60 seconds. Wash in TBS wash buffer.
7**. Blocking Step (Optional):**
- Apply Rodent Block M for 30 minutes Wash in TBS buffer.
8. Apply primary antibody for 30-60 minutes or overnight at 2-8ºC. Wash in TBS wash buffer. (See Technical Note #4).
9. Apply the Rat Probe for 10-15 minutes. Wash in TBS wash buffer.
10. Apply the Rat-on-Mouse AP-Polymer for 10-15 minutes. Wash in TBS wash buffer (See Technical Note #5)
11. Chromogen: Incubate for 5-7 minutes when using Warp Red. Rinse in DI water.
12. Counterstain with hematoxylin. Wash in DI water. Apply Tacha's Bluing Solution for 1 minute. Wash in DI water.
13. Dehydrate, clear and coverslip.

Technical Notes:
1. Post-fixing tissue sections on slides for 15-30 minutes in 10% formalin reduces endogenous mouse IgG and helps prevent tissues from falling off the slides.
2. Blocking for endogenous peroxidase is not required; however, immersing slides in Peroxidazed 1 bleaches tissues, red blood cells and produces better contrast for alkaline phosphatase staining procedures.
3. This product is designed to work with 1X Rodent Decloaker. Other antigen retrieval solutions and/or protocols may cause non-specific background or insufficient staining.
4. Background Punisher can be used as a blocker for nonspecific background staining.
5. If endogenous mouse IgG is observed in tissue sections, add 1-2 drops (1 drop equals 33µL) of XM Factor to 2.5 mL of Rat-on-Mouse AP- Polymer.
6. Use TBS wash buffer only. PBS wash buffer will inhibit alkaline phosphatase staining.
7. Biocare Medical has novel products to help eliminate persistent mouse IgG. XM Factor is a very potent blocker for eliminating endogenous mouse IgG. By adding 1-2 drops (1 drop equals 33µL) of the XM Factor to 2.5mL of the Rat-on-Mouse Polymer, endogenous mouse IgG will be dramatically reduced and/or completely eliminated. Rodent Block M is a blocking reagent for nonspecific background staining that also reduces endogenous mouse IgG. Rodent Block M is applied prior to the primary antibody for 15-30 minutes.

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Limitations:
This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

Precautions:
1. Kit reagent(s) contain 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)[1]
2. 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.
3. 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.[2,3]
4. 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.[4]
5. Microbial contamination of reagents may result in an increase in nonspecific staining.
6. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
7. Do not use reagent after the expiration date printed on the vial.
8. The reagent is optimized for 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.
9. Follow local and/or state authority requirements for method of disposal.
10. The SDS is available upon request and is located at http://biocare.net.

This micro-polymer detection kit contains components classified as indicated in the table below in accordance with the Regulation (EC) No. 1272/2008.

Hazard	Code	Hazard Statement
	H317	May cause an allergic skin reaction.
N/A	H402 H412	Harmful to aquatic life. Harmful to aquatic life with long lasting effects.

Technical Support:
Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

Troubleshooting Guide:
No Staining
1. Critical reagent (such as primary antibody) omitted.
2. Staining steps performed incorrectly or in the wrong order.
3. Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, the wrong order, or the wrong pretreatment.
4. Insufficient amount of antigen.
5. Primary antibody incubation period too short.
6. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining
1. Tissue is either over-fixed or under-fixed.
2.Primary antibody incubation too short.
3. Low expression of antigen.
4.Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment.
5. Over-development of substrate.
6. Excessive rinsing during wash steps.
7. Omission of critical reagent.
8. Incorrect procedure in reagent preparation.
9. Improper procedure in test steps.

Non-specific or High Background Staining
1. Tissue is either over-fixed or under-fixed.
2. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
3. Tissue may need a longer or a more specific protein block.
4. Substrate is overly developed.
5. Tissue was inadequately rinsed.
6. Deparaffinization incomplete.
7. Tissue damaged or necrotic.

Tissues Falling Off
1. Slides were not positively charged.
2. A slide adhesive was used in the water bath.
3. Tissue was not dried properly.
4. Tissue contained too much fat.

Specific Staining Too Dark
1. Concentrated antibody not diluted out properly (being used at too high of a concentration).
2. Incubation of primary antibody or detection too long.

References:
1. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts.
2. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
3. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
4. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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