

Goat-on-Rodent AP-Polymer

Goat Primary Antibodies on Mouse, Rat and Human Tissues
Polymer Detection Component
902-GAP514-080217

BIOCARE
M E D I C A L

Catalog Number: GAP514 G, H

Description: 6, 25 ml

Intended Use:

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

Summary & Explanation:

Goat-on-Rodent Alkaline Phosphatase (AP) polymer has been developed for use with goat primary antibodies on mouse, rat and human tissues. Goat primary antibodies are advantageous because goat secondary detections display minimum cross-reactivity to endogenous mouse, rat, and human IgG. Advantages of Biocare Medical's biotin-free polymer technology are increased sensitivity, a reduction of IHC steps and elimination of endogenous biotin.

It can be used with formalin-fixed paraffin-embedded tissues, floating sections, frozen sections or cell cultures. The Goat-on-Rodent AP-Polymer is comprised of two reagents: the goat probe that labels the goat primary antibody and an AP-polymer that conjugates to the probe. This two-step system has been proven to be 10-20 times more sensitive than conventional mouse anti-goat AP secondary detection systems.

Biocare Medical has also developed Rodent Decloaker, an antigen retrieval solution, which helps reduce and eliminate endogenous mouse and rat IgG and non-specific background staining while simultaneously performing antigen retrieval. For human tissues, please refer to the respective primary antibody data sheet for recommended solutions. Temperature dependent protocols can be performed using Biocare's Decloaking Chamber.

Known Applications:

Immunohistochemistry (Formalin-fixed paraffin-embedded tissues)

Supplied As:

6 ml Kit

Goat Probe (AP) (GP613G) 6ml

Goat-on-Rodent AP-Polymer (GAP628G) 6ml

25 ml Kit

Goat Probe (AP) (GP613H) 25ml

Goat-on-Rodent AP-Polymer (GAP628H) 25ml

Materials and Reagents Needed But Not Provided:

Microscope slides, positively charged

Desert Chamber* (Drying oven)

Positive and negative tissue controls

Xylene (Could be substituted with xylene substitute*)

Ethanol or reagent alcohol

Decloaking Chamber* (Pressure cooker)

Deionized or distilled water

Wash buffer*

Pretreatment reagents*

Enzyme digestion*

Peroxidase block*

Protein block*

Primary antibody*

Negative control reagents*

Chromogens*

Hematoxylin*

Bluing Reagent*

Mounting medium*

* Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Species Reactivity:

Goat IgG heavy and light chains with minimal cross reactivity on mouse, rat and human tissues.

Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user.

Staining Protocol Recommendations:

1. Deparaffinize slides in Biocare's Slide Brite or xylene.

2. Hydrate slides in a series of graded alcohols to water.

3. **Optional:** Post-fix rodent tissues in 10% formalin for 30 minutes. Wash in DI water. (See Technical Note #4)

4. **Optional:** Immerse slides in Biocare's Peroxidized 1 blocking reagent for 3-5 minutes. Wash in DI water. (See Technical Note #1)

5. For rodent tissues, place slides in 1X Rodent Decloaker and heat in Biocare's Decloaking Chamber. Heating times can be used as follows:

- 80°C for 30-120 minutes or overnight for 12 to 18 hours

- 95°C for 30-60 minutes

- 110°C for 15 minutes

- 125°C for 30 seconds or 5 minutes at high temperature

Remove slides and wash in DI water.

For human tissues please refer to the respective primary antibody data sheet for recommended solutions and protocol.

6. Digestion Technique (Optional):

- Place rodent tissue slides in 1X 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 necessary, apply pepsin at (RT) for 10-15 minutes and wash in DI water.

- If using Rodent Decloaker at 95°C to 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 Background Punisher (human tissue) for 10 minutes. Wash in TBS buffer. (See Technical Note #2)

8. Apply primary antibody for 30-60 minutes at RT or overnight at 2-8°C. Wash in TBS wash buffer.

9. Apply Goat Probe (AP) for 10-15 minutes. Wash in TBS wash buffer.

10. Apply Goat-on-Rodent AP-Polymer for 10-15 minutes. Wash in TBS wash buffer.

11. **Chromogen:** Incubate for 5-7 minutes when using Biocare's 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. Blocking for endogenous peroxidase is not required; however, immersing slides in Peroxidized 1 bleaches tissues and red blood cells which produces better contrast for alkaline phosphatase staining procedures.



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Technical Notes Cont'd:

2. Biocare's Background Punisher can be used as a blocker for non-specific background staining.
3. Use TBS wash buffer only. PBS wash buffers will inhibit alkaline phosphatase staining.
4. Post-fixing rodent tissue sections on slides for 15-30 minutes in 10% formalin reduces endogenous mouse and rat IgG and helps prevent tissues from falling off the slides.

Protocol Notes:

N/A

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.

Covered by one or more of the following US Pat. Nos. 6,686,461; 6,800,728; 7,102,024; 7,173,125; 7,462,689.

Precautions:

1. This product is not classified as hazardous. The preservative used in this reagent is Proclin 300 and the concentration is less than 0.25%. Overexposure to Proclin 300 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 300 in this product does not meet the OSHA criteria for a hazardous substance. Wear disposable gloves when handling reagents.
2. 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 in contact with sensitive areas, wash with copious amounts of water.
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.
7. Consult OSHA, federal, state or local regulations for disposal of any toxic substances. Proclin™ is a trademark of Rohm and Haas Company, or of its subsidiaries or affiliates.

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. Endogenous alkaline phosphatase (not blocked with levamisole).
3. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
4. Tissue may need a longer or a more specific protein block.
5. Substrate is overly-developed.
6. Tissue was inadequately rinsed.
7. Deparaffinization incomplete.
8. Tissue damaged or necrotic.

Tissues Falling Off

1. Slides were not positively charged.
2. A slide adhesive was used in the waterbath.
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.