# Goat-on-Rodent HRP-Polymer

Goat Primary Antibodies on Mouse, Rat and Human Tissues
Polymer Detection Component
902-GHP516-080717

BIOCARE MEDICAL

**Catalog Number:** **GHP516 G, H, L**

**Description:** 6.0, 25, 100 ml

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

**Summary & Explanation:**
Goat-on-Rodent Horseradish Peroxidase (HRP) 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 HRP-Polymer is comprised of two reagents: the goat probe that labels the goat primary antibody and an HRP-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 HRP 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:**
**6ml**
Goat Probe (HRP) (GP626G) 6ml
Goat-on-Rodent HRP-Polymer (GH627G) 6ml
**25ml**
Goat Probe (HRP) (GP626H) 25ml
Goat-on-Rodent HRP-Polymer (GH627H) 25ml
**100ml**
Goat Probe (HRP) (GP626L) 100ml
Goat-on-Rodent HRP-Polymer (GH627L) 100ml

**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 2ºC to 8ºC. 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.

**<u>Staining Protocol Recommendations:</u>**
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 #1)
4. Immerse slides in Biocare's Peroxidazed 1 blocking reagent for 3-5 minutes. Wash in DI water.
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 - 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 solution 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. 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 2-8ºC. Wash in TBS wash buffer.
9. Apply Goat Probe (HRP) for 10-15 minutes. Wash in TBS wash buffer.
10. Apply Goat-on-Rodent HRP-Polymer for 10-15 minutes. Wash in TBS wash buffer.
11. **Chromogen:** Apply DAB for 5 minutes. 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.

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**Technical Notes:**
1. 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 slides.
2. Biocare's Background Punisher can be used as a blocker for nonspecific background staining.

**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.

**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. 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 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.

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