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Super Clean Background, High Sensitivity, Biotin-Free Technology

IMMUNOHISTOPROBE

Anti-Mouse HRP Polymer Detection Kit

(Made in Goat, min. cross with Hm.&Rbt.)

(For IHC Detection **Mouse** Primary antibody)

Catalogue Number: **1811-07L (125ml, w/o chromogen)**
1811-07MD (60ml, w/DAB)
1811-07SD (18ml, w/DAB)

Product Information:

Product Description: ImmunoHistoProbe Anti-Mouse HRP Polymer detection kit (made in Goat)
Host species: Goat
Specificity: Mouse IgG (H+L)
Cross Reactivity: Minimal cross reactivity with human and rabbit serum proteins.
Preservative: Proclin 300
Conjugated enzyme: Polymerized Horseradish Peroxidase
Application: Immunohistochemistry for frozen and paraffin embedded tissues.

Intended Use:

For In vitro Laboratory Use Only. Not for diagnostic or therapeutic use.

ImmunoHistoProbe anti-Mouse HRP Polymer Detection Kit is specifically designed for visualization of mouse primary antibodies on human tissue. The tissue sample can be paraffin-embedded tissue section, frozen sections and cell preparations. It is for research use only.

Background Introduction:

ImmunoHistoProbe One Step anti-Mouse HRP Polymer Detection Kits utilized the newest biotin-free polymerization technology to prepare super sensitive polymeric peroxidase linked conjugates. The labeled polymer does not contain avidin or biotin, thus, non-specific staining caused by endogenous biotin is completely eliminated. It provides one step detection protocol, superior sensitivity and specificity, short incubation time and faster turnaround.

Prior to staining the formalin-fixed paraffin tissue sections should be deparaffinized and hydrated following heat-induced epitope retrieval or enzyme pretreatment, if necessary. Endogenous peroxidase should be blocked. Then add primary antibody and incubate at optimal titration and condition. After labeling the antigen on the tissue or cell preparations with primary antibody, add affinity-purified polymer HRP conjugated secondary for 10-15 minutes. Polymer HRP enzyme will catalyze the substrate/chomogen, 3,3' diaminobenzidine (DAB) or 3-Amino-9-ethylcarbazole (AEC) reaction to form visible brown (DAB) or red color (AEC) deposit at the antigen site. The antigen then can be visualized under microscope. When color development achieved satisfactory level, the slides are washed in H_2O to stop reaction. The stained slides may be mounted with either aqueous mounting medium or organic mounting medium.

Reagent Provided:

The volume of each reagent provided in the kits is listed below:

Reagent Included	Format	1811-07L	1811-07MD	1811-07SD
Reagent 1: HRP Polymer anti-Mouse (ready to use)	Liquid with red color	1x125ml	1 x 60ml	1 x 18ml
Reagent 2A: DAB chromogen (20x concentrated)	Liquid with light to dark brown color	Not included	1x3ml	1x1ml
Reagent 2B: DAB substrate buffer	Clear Liquid	Not included	1x60ml	1x18ml

HRP Polymer anti-Mouse is pre-diluted and ready to use. Reconstitution, mixing, dilution or titrations of these reagents are not recommended. Further dilution may result in loss of antigen staining signal.

Materials required but not supplied:

1. Standard solvents used in immunohistochemistry.
2. Wash buffer (Recommend: 50mM Tris-buffered saline, pH 7.6 or 10mM PBS buffer pH 7.4, with 0.05% Tween-20).
3. Antigen Retrieval buffer (optional, necessary if primary antibody request).
4. Enzyme Retrieval buffer (optional, necessary if primary antibody request).
5. Primary antibody and diluent.
6. Mounting medium.
7. General Immunohistochemistry laboratory equipment and consumables.

Storage and Stability:

Store at 2-8^{0}C. Do not freeze. Return to 2-8^{0}C immediately after use. Check expiration date on bottle. Do not use the reagents if the expiration dates on the label have passed. Do not mix the reagents from different lot. Since there are no obvious signs to

indicate the instability of this product therefore positive and negative controls should be run simultaneously with test specimens.

Warnings and Precautious:

1. For professional users only.
2. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
3. Specimens, before or after fixation and all materials exposed to them, should be handled as if infectious and disposed of with proper precautions.
4. Do not substitute reagents from other lot numbers or from kits of other manufacturers.
5. Incubation times or temperatures other than recommendation must be validated by the user.
6. Unused solution should be disposed of according to local, State and Federal regulations.
7. The Safety Data Sheet is available upon request or available from http://www.advancedbiosystemsinc.com.

Protocol Recommendations:

Users must be trained in immunohistochemical technique prior to undertaking the following protocol.

Specimen Preparations:

For use with formalin-fixed, paraffin-embedded tissue sections as well as frozen tissue sections and cell smears.

Prior to IHC staining, appropriate tissue fixation and processing are required to obtain optimum performance and reliable interpretations. Optimal fixatives and procedures need to be determined and verified by the user. Cell smears prepared from body fluids should be a monolayer of cells. Smears should be fixed immediately after preparation. Fixation of frozen or cytospin sections can be accomplished with 100% acetone or methanol at 4°C for 10 minutes.

Tissue Preparations:

For formalin-fixed, paraffin-embedded tissue sections: cut and mount sections on slides coated with suitable tissue adhesive. Drain excess water from the slides. Dry tissue according to general protocol. Deparaffinize sections in xylene or xylene substitutes with 2 changes for 5 minutes each. Rehydrate through graded alcohol (100%, 95% and 70%). Rinse slides with distilled water.

Control slides are needed for proper interpretation of each set of specimen staining results: positive tissue control, negative tissue control and negative reagent control (slide treated with isotype control in place of primary antibody).

IHC Staining Procedures:

Do not allow tissue sections to dry during the staining procedure. Dried tissue sections may show increased nonspecific staining. If prolonged incubations are needed, place tissues in a humidity chamber.

Step 1: Block Endogenous Peroxidase Activity

For paraffin sections-incubate sections with 3% hydrogen peroxide for 10 minutes. Then rinse in distilled water.
For frozen sections: incubate sections with 0.3% hydrogen peroxide in methanol for 20-30 minutes. Rinse with distilled water.

Step 2: Antigen Retrieval (optional)

Perform heat induced antigen retrieval or enzyme pretreatment as required. The user needs to optimize the antigen retrieval condition for each primary antibody.

Step 3: Primary Antibody or Negative Control Reagent

Add enough optimally diluted primary antibody or negative control reagent to cover tissue sections. Incubate under optimal temperature and length of time. The user needs to validate the best condition for each primary antibody. Rinse 3 x 2 minutes in wash buffer.

Step 4: HRP Polymer Goat anti-Mouse

Add enough ready-to-use ***Reagent 1 (HRP Polymer Anti-Mouse,*** *ready to use****)*** to cover tissue sections completely. Incubate 10-15 minutes. Rinse in wash buffer for 3 x 2 minutes.

Step 5: Chromogen (not included in 125ml size kit, recommend DAB Chromogen Kit Cat No. DAB-125 or Cat No. DAB-60.)

Prepare DAB working solution: Add one drop or 50ul ***Reagent 2A (DAB chromogen****, 20x concentrated)* into 1ml ***Reagent 2B (DAB substrate buffer****, 1x).* User may make any amount of DAB working solution using the same ratio (1:20). Use within 2 hours after preparation.
Apply enough of DAB working solution to cover the specimen completely. Incubate 5-10 minutes. Monitor the color development under light microscope. Rinse slides gently with distilled water.

Step 6: Counterstain and mounting.

Counterstain, clear, and mount in appropriate mounting medium.

Troubleshooting:

No Staining on positive slides:

Staining steps were performed incorrectly; Primary or secondary antibody incubation was omitted; Specimen dehydrated during staining; Heat-induced epitope retrieval (HIER) was insufficient or omitted; Insufficient amount of antigen; etc.

Weak Staining on all slides:

Incubation of primary antibody may be too short; Tissue may be over-fixed or poorly processed; Low expression of antigen; Substrate prepared improperly; etc.

Non-specific or High Background Staining

Endogenous enzyme activity was incompletely blocked; Deparaffinization was incomplete; Inadequate rinse of slides; Dehydration of specimen during staining. Different block buffer may be needed; Over-development of substrate; Excessive tissue adhesive; primary antibody too concentrated; etc.

Staining on negative control
Secondary antibody may contain cross-reactive antibodies; Inadequate blocking for endogenous peroxidase; Tissue may contain endogenous pigment; Tissue may be necrotic; etc.

Limitations and warranty:

Immunohistochemistry is a multistep process and good results will depend on the proper handling and processing of the tissue both prior to and during 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. Our warranty is limited to the actual price paid for the product. We are not liable for any property damage, personnel injury, time, effort or economic loss due to use our product.

REFERENCES:

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