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9. Disclaimer

INTENDED USE: For Research Use Only.

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GeneAb™

Anti-Mouse/Rabbit 2 Step Polymer Detection Kit (w/ DAB)

Research Use Only

1. Introduction

GeneAb™ Anti-Mouse/Rabbit HRP Polymer Detection Kit utilizes the newest biotin-free polymerization technology to prepare highly sensitive polymeric peroxidase linked conjugates. It is based on signal amplification by using a two-step polymer detection method. The primary antibody specific to an antigen on the tissue section is detected by an amplifier for the primary antibody, followed by polymer detector. The antigen sites are then visualized with chromagen, DAB (3,3'-Diaminobenzidine). Comparing to the conventional labeled Streptavidin-Biotin or Avidin Biotin Complex methods, GeneAb™ Anti-Mouse/Rabbit HRP Polymer Detection Kit completely eliminates the potential background caused by endogenous biotin activity on biotin-rich tissues. GeneAb™ anti-Mouse/Rabbit HRP Polymer Detection Kit also uses new polymerization technology that allows a larger number of peroxidase enzyme to be coupled with secondary antibody, increasing the sensitity dramatically.

2. Kit Components

REF R020

PN Part Number	Kit Components	Quantity
R020- A	Amplifier For Mouse/Rabbit (ready to use)	1 x 20 ml
R020-B	HRP Polymer Detector (ready to use)	1 x 20 ml
R020-C	DAB substrate buffer	1 x 20 ml
R020-D	DAB chromogen (20x concentrated)	1 x 1 ml

REF R100

PN Part Number	Kit Components	Quantity
R100- A	Amplifier For Mouse/Rabbit (ready to use)	1 x 100 ml
R100-B	HRP Polymer Detector (ready to use)	1 x 100 ml
R100-C	DAB substrate buffer	1 x 100 ml
R100-D	DAB chromogen (20x concentrated)	1 x 5 ml

3. Storage and Stability

Store at 2-8°C. Do not freeze. Return to 2-8°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.

4. Precautions

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.genomeme.ca

5. Application Summary

Prior to staining and if deemed necessary, the formalin-fixed paraffin tissue sections should be deparaffinized and hydrated following heat-induced epitope retrieval or enzyme pretreatment. Endogenous peroxidase should also be blocked. Add your primary antibody and incubate at optimal titration and conditions. After labeling the antigen on the tissue or cell preparations with your primary antibody, apply R020-A/R100-A (Amplifier for Mouse/Rabbit, ready to use) and incubate for 10-15 minutes. Afterwards, apply R020-B/R100-B (HRP Polymer Detector, ready to use) for 10-15 minutes. Finally, add R020-D/R100-D (DAB chromogen, 20x concentrated) to form visible brown deposit at the antigen site. The antigen then can now be visualized under the microscope. When the color development is achieved to a satisfactory level, wash the slide with distilled water to stop the reaction. The stained slide may be mounted with either aqueous mounting medium or organic mounting medium.

6. Protocol

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. Rinse with 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: Amplifier for Mouse/Rabbit
Add enough ready-to-use R020-A/R100-A (Amplifier for Mouse/Rabbit, ready to use) to cover tissue sections completely. Incubate 10-15 minutes. Rinse in wash buffer for 3 x 2 minutes.

Step 5: HRP Polymer Detector
Add enough ready-to-use R020-B/R100-B (HRP Polymer Detector, ready to use) to cover tissue sections completely. Incubate 10-15 minutes. Rinse in wash buffer for 3 x 2 minutes.

Step 6: Chromogen
Prepare DAB working solution: Add one drop or 50ul R020-D/R100-D (DAB chromogen, 20x concentrated) into 1ml R020-C/R100-C (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 7: Counterstain and mounting.
Counterstain, clear, and mount in appropriate mounting medium.

7. 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.

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.

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.

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.

8. References

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