

MaxHomo™ Mouse on Mouse Polymer AP Detection Kit (w/AP-Red)

(For IHC detection of Mouse primary antibodies on Mouse tissue)
Super Clean Background, High Sensitivity, Biotin-Free Technology

Catalog Number:

Cat No.	Description	For Primary Antibody	Enzyme	Size	Good For**
HM02-A	MaxHomo™ Mouse on Mouse Polymer AP Detection Kit (18ml, w/ AP-Red)	Mouse	AP	18ml	180 slides
HM02-AS	MaxHomo™ Mouse on Mouse Polymer AP Detection Kit (6ml, w/ AP-Red)	Mouse	AP	6ml	60 slides

**Number of good for slides is calculated based on adding 100ul per slide.

Intended Use:

MaxVision's MaxHomo™ Mouse on Mouse Polymer AP Detection Kit is designed specifically to localize mouse primary antibodies on mouse tissue. The tissue sample can be paraffin-embedded mouse tissue section, frozen sections and cell preparations.

Known applications: Immunohistochemistry

It is for research use only.

Introduction:

MaxHomo™ Mouse on Mouse Polymer HRP (or AP) Detection Kits are designed to localize mouse primary antibody on mouse tissue. The major problem encountered by the investigators when they try staining mouse primary antibodies on mouse tissue using indirect method is the inability of anti-mouse secondary antibody to distinguish between endogenous mouse immunoglobulins and mouse primary antibody. This problem will cause high background staining while the specific staining is then obscured. This same problem will happen when try to detect other antibodies on homologous tissues. MaxHomo™ blocking reagent is special designed and formulated to block endogenous immunoglobulins which lead to the most complete elimination of background staining. MaxHomo™ Mouse on Mouse Polymer Detection kit utilizes the newest polymerization technology to achieve the highest sensitivity. Since it is biotin-free the endogenous biotin background in some biotin-rich tissue is completely eliminated. Using MaxHomo™ Mouse on Mouse Polymer HRP (or AP) Detection Kit the user may expect the best signal-to-noise ratio comparing to any other kits on the market.

Prior to staining formalin-fixed paraffin tissue sections should be deparaffinized and hydrated following heat-induced epitope retrieval or enzyme pretreatment. Endogenous peroxidase should be blocked. Then apply serum-free protein block for blocking nonspecific background. After protein blocking incubate specimen in MaxHomo™ blocking reagent which binds to endogenous Igs then primary antibody is added following post primary blocking. For the best staining signal mouse antibody signal amplifier is then added before polymer HRP (or AP) secondary antibody incubation. MaxHomo™ mouse on mouse polymer kits used the newest polymerization technology which allows a larger number of peroxidase (or alkaline phosphatase) coupled with secondary antibody. This technology increases the sensitivity dramatically. The optimal dilutions and incubation times for primary antibodies may need to be re-adjusted. 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. Polymer AP enzyme will catalyze the substrate/chomogen such as Fast-Red, AP-Red, AP-Blue, or BCIP/NBT to form a visible red (Fast-Red or AP-Red) or dark blue/purple (BCIP/NBT) or blue (AP-Blue) color deposit at the antigen site. The antigen then can be visualized under microscope. When color development achieved satisfactory level, the slides are washed in H₂O to stop reaction. The stained slides may be mounted with either aqueous Easy Mount (Cat No EZM-L, EZL-M) or organic mounting medium Eco-O-Mount (Cat No. EOM-L or EOM-M).

MaxHomo™ reagents are provided in ready-to-use format for user's convenience.

Reagent Provided:

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

Reagent Descriptions	\ Cat No.	HM02-A	HM02-AS
Reagent 1: Protein Blocking Solution (ready to use)		1 x 18ml	1 x 6ml
Reagent 2: MaxHomo™ Blocking Reagent (ready to use)		1 x 18ml	1 x 6ml
Reagent 3: Post Primary Blocking (ready to use)		1 x 18ml	1 x 6ml
Reagent 4: Antibody Signal Enhancer (ready to use)		1 x 18ml	1 x 6ml
Reagent 5: Polymer AP Secondary Antibody (ready to use)		1 x 18ml	1 x 6ml
Reagent 6A: AP-Red enhancer (40x)		1 x 2ml	1 x 1ml
Reagent 6B: AP-Red Concentrate (40x)		1 x 2ml	1 x 1ml
Reagent 6C: Substrate Solution Concentrate (20x)		1 x 3ml	1 x 2ml

1. Reagent 1 Protein Blocking Solution is animal serum free solution in phosphate-buffered saline with stabilizer, surfactant and preservative.
 2. Reagent 2 MaxHomo™ Blocking Reagent is proprietary formulated solution in buffer with preservative.
 3. Reagent 3 Post Primary Blocking in phosphate buffer saline with preservative.
 4. Reagent 4 Antibody Signal Enhancer containing stabilizer and preservative.
 5. Reagent 5 Polymer AP Secondary Antibody is formulated in stabilizing solution with anti-microbial agent.
 6. Reagent 6A: AP-Red enhancer (40x)
 7. Reagent 6B: AP-Red Concentrate (40x)
 8. Reagent 6C: Substrate Solution Concentrate (20x)
- Protein Blocking Solution, MaxHomo™ Blocking Reagent, Post Primary Blocking, Antibody Signal Enhancer and Polymer AP Secondary Antibody are prediluted. 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).
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. Counterstain reagent.
7. Mounting medium. (Recommend: Easy-Mount (Cat No. EZM-L, EZL-M)).
8. General Immunohistochemistry laboratory equipment and consumables.

Storage and Stability:

Store at 2-8°C. Do not freeze. Return to 2-8°C immediately after use. 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 Precautions:

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

Protocol Recommendations:

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 Alkaline Phosphatase Activity (optional, do this step if elimination of endogenous alkaline phosphatase activity is necessary).

For endogenous intestinal alkaline phosphatase activity, user may treat the sections in 20% acetic acid at 4°C for 15 minutes or with 2.3% periodic acid for 5 minutes then 0.02% potassium borohydride for 2 minutes.

For non-intestinal alkaline phosphatase, adding levamisole to the substrate solution can inhibit its activity.

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. Suggest products: Enzyme pretreatment solution (Trypsin Cat No. E01-L, E01-M; Pepsin Cat No. E02-L, E02-M; Pronase kit Cat No. E03-L, E03-M).

Step 3: Protein Blocking Solution

Add enough (about 1-3 drops) Protein Blocking Solution (**Reagent 1**) to completely cover tissue sections. Incubate tissue sections for 10 minutes. Blot excess blocking reagent from sections. DO NOT RINSE.

Step 4: MaxHomo™ Blocking Reagent

Add enough MaxHomo™ Blocking Reagent (**Reagent 2**) to completely cover tissue sections. Incubate tissue sections for 30 minutes. Rinse 3 x 2 minutes in wash buffer.

Step 5: 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 6: Post Primary Blocking

Add enough Post Primary Blocking (**Reagent 3**) to completely cover tissue sections. Incubate tissue sections for 30 minutes. Rinse 3 x 2 minutes in wash buffer.

Step 7: Antibody Signal Enhancer

Add enough ready-to-use Antibody Signal Enhancer (**Reagent 4**) to cover tissue sections completely. Incubate 20 minutes. Rinse in wash buffer for 3 x 2 minutes.

Step 8: Polymer AP Secondary Antibody

Add enough ready-to-use Polymer AP Secondary Antibody (**Reagent 5**) to cover tissue sections completely. Incubate 20 minutes. Rinse in wash buffer for 3 x 2 minutes.

Step 9: Chromogen

Make AP-Red working solution: Add 1 drop of AP-Red enhancer (**Reagent 6A**) and 1 drop of AP-Red concentrate (**Reagent 6B**) to a test tube; Mix well and incubate for 2-5 minutes; Add 2 ml distilled water. Mix well; Add 2 drops of Substrate Solution Concentrate (**Reagent 6C**) to the mixture; Mix well. Apply enough of AP-Red working solution to cover the specimen completely. Allow 10-30 minutes, under room temperature, for the color to develop. Monitor the color development under light microscope. Rinse slides gently with distilled water to stop the reaction.

Step 10: Counterstain and mounting.

Counterstain, and mount in appropriate mounting medium. AP-Red is alcohol soluble. DO NOT Dehydrate. Use aqueous mounting medium. Recommend: Easy-Mount (Cat No. EZM-L, EZL-M).

Troubleshooting:

Results	Possible causes:
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 alkaline phosphatase; 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.

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