MedaView™ One-step Polymer-AP Anti-Mouse&Rabbit System

Intended Use: For Research Use Only. This kit is intended for use in immunohistochemistry (IHC) staining protocols on formalin-fixed, paraffin- embedded tissue (FFPE) sections.

Description: MedaView™ One-step Polymer-AP Anti-Mouse&Rabbit System is a biotin-free visualization system used in immunohistochemical protocols. It is an indirect method designed based on proprietary technologies to detect mouse and rabbit primary antibodies on the targeted specimens. The resulting antibody-enzyme can be visualized by AP-compatible chromogens and observed by bright field microscopy. This method increases staining sensitivity when compared to other conventional AP detection methods and eliminates non-specific staining caused by endogenous biotin. This detection system is compatible with both manual and open automated staining platforms.

Package:

Description	Catalog No.	Size
MedaView™ One-step Polymer-AP Anti-Mouse&Rabbit System	DP1120	15ml
MedaView™ One-step Polymer-AP Anti-Mouse&Rabbit System	DP1121	100ml

Reagents Supplied: One bottle of ready-to-use MedaView™ One-step Polymer-AP Anti-Mouse&Rabbit reagent

Storage and Stability: Store at 2°- 8°C. Do not freeze. All performance claims are void after the kit expiration date.

Materials Required but Not Supplied:
Xylene, graded EtOH, antigen retrieval solutions, peroxide block, negative control reagents, primary antibody, chromogen & substrate, IHC wash buffer, hematoxylin, mounting medium, graduated cylinder, microscope slides, drying oven, positive and negative controls, slide rack, water bath, microwave or pressure cooker.

Recommended Staining Protocol:
1. Dewax FFPE sections in xylene or xylene substitute. Rehydrate sections in a series of graded EtOH to water.
2. Perform antigen retrieval or protease pretreatment according to manufacturer's datasheets for primary antibodies.
3. Apply peroxide block for 5-10 minutes at RT; rinse with IHC wash buffer.
4. Apply primary antibody according to manufacturer's datasheet; rinse with IHC wash buffer.
5. Apply MedaView™ One-step Polymer-AP Anti-Mouse&Rabbit for 20 minutes at RT; rinse with IHC wash buffer.
6. Apply AP-compatible chromogen according to manufacturer's datasheet; rinse with distilled or deionized water.
7. Counterstain with hematoxylin or other compatible counterstain solutions for 1-2 minutes; wash with running tap water.
8. Dehydrate in graded EtOH series for 2 minutes (70%, 85%, 95%, 100%, 100%).
9. Immerse in Xylene 2 times, 2 minutes each.
10. Coverslip.

Quality Control:
Refer NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on Tissue Controls.

Limitations:
The protocols involve multiple factors and steps that require trained lab professionals to operate. It is the responsibility of the investigator to determine optimal conditions.

Precautions:
This product is not classified as hazardous. Refer to MSDS.
Specimens and materials exposed to them should be regarded as potentially infectious and properly disposed of. Wear disposable gloves when handling reagents. 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. Microbial contamination of reagents may produce incorrect results.
Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

© Medaysis Company

Troubleshooting:
No Staining or Weak Staining
1. Low expression of antigen or loss of antigenic differentiation. Use a more sensitive detection system (two-step polymer system) and/or re-optimize protocols.
2. Tissue under-fixed. Increase duration of postfixation or try different fixatives.
3. Excessive cross-linking and reduction in antigen availability caused by over-fixation. Decrease duration of postfixation or perform appropriate antigen retrieval.
4. Inadequate dewaxing. Increase dewaxing time or use fresh xylene.
5. Insufficient antigen retrieval, Optimize the antigen retrieval protocol including proper pH of solutions, and temperature.
6. Reduced sensitivity of primary antibody caused by inappropriate diluent. Check formula and make sure the diluent has the stabilizing protein, avoid excessive NaN3 and changes of ion and pH value.
7. Antibody incubation time too short. Increase incubation time.
8. Too low concentration of primary or secondary antibody. Increase the concentration or run a serial dilution test to determine the optimal dilution for the best signal to noise ratio.
9. Inactive critical reagents. Replace with a new batch and avoid repeated freeze and thaw cycles. Or store antibodies according to manufacturer's datasheets.
10. Incorrect mounting medium, i.e. alcohol-based mounting media will remove aqueous-based chromogens. Use aqueous-based mounting media for aqueous-based chromogens, use alcohol-based mounting media for permanent chromogen.
11. Reagents applied in wrong order or steps omitted. Check notes or procedure used.

High Background
1. Diffusion of tissue antigen due to inadequate fixation. Increase duration of postfixation or fix smaller pieces.
2. Incomplete dewaxing. Optimize the dewaxing step or use fresh xylene.
3. Sections dried out. Avoid sections being dried out.
4. Presence of endogenous peroxidase or alkaline phosphatase. Block endogenous enzyme activities using 3% hydrogen peroxide (block peroxidase) in methanol or levamisole solution (block AP) prior to incubation of primary antibodies.
5. Presence of endogenous biotin. Block endogenous biotin activity using the avidin/biotin blocking reagent prior to incubation of primary antibodies.
6. Incubation time of primary and secondary antibody was too long. Reduce incubation time.
7. Non-specific binding of primary antibodies to tissue or antibody concentration too high. Reduce the concentration or run a serial dilution test to determine the optimal dilution for the best signal to noise ratio.
8. Cross reactivity of secondary antibodies with tissue of close species, i.e. rabbit anti-rat IgG may cross react with mouse tissue. Pre-adsorb secondary antibody with species-specific normal serum from tissue, i.e. absorb rabbit anti-rat IgG with mouse normal serum before using on mouse tissue.
9. Mouse antibodies used on mouse tissues. Use Mouse on Mouse blocker prior to the primary antibody incubation.
10. Substrate over developed. Reduce incubation time.
11. Insufficient washing. Wash at least 3 times between steps.

Tissues Falling-off
1. Unfixed or under-fixed tissues tend to come off slides. Increase fixation time or duration of postfixation.
2. Formaldehyde fixed frozen sections tend to fall off slides. Dry slides longer or use alternative fixation such as acetone.
3. Uncharged or uncoated slides used. Use positively charged or gelatin coated slides.
4. Waterbath contaminated by hand lotion. Always wear gloves.
5. Disposable blades covered by oil. Clean disposable blades with xylene.
6. Wrinkles in the sections during the initial mounting. Spread out the sections on slides and avoid wrinkles.
7. Sections dried incompletely before placing in the oven (FFPE) or before fixation and staining (frozen sections). Air dry sections a minimum of 30 minutes before placing in the oven at 56°C overnight (FFPE), or a minimum of 30 minutes before fixation and then another 30 minutes before staining (frozen sections).
8. High pH antigen retrieval solutions such as EDTA pH8.0 or Tris-EDTA pH9.0 used. Used low pH solutions such as citrate pH6.0 or enzyme digestion if possible.
9. Distilled water used for washing. Always use buffer solution to wash or rinse slides.
10. Pressure cooker or microwave may be the cause. Use alternative device such as waterbath or steamer for antigen retrieval.
11. Plain water in waterbath for mounting FFPE sections. Add gelatin in waterbath.

Orders: customercare@medaysis.com Support: techsupport@medaysis.com Tel: 510-509-3153 www.medaysis.com
© Medaysis Company