Product Manual

MULTIVIEW® (mouse-HRP/mouse-AP) IHC kit

Catalog #: ADI-950-101

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTENDED USE

The MULTIVIEW® (mouse-HRP/mouse-AP) IHC kit is a non-biotin, one-step detection system suitable for demonstrating antigens in formalin-fixed paraffin-embedded tissues and cryostat sections. It may also be used with blood smears, cytosmears, and cell preparations.

The MULTIVIEW® (mouse-HRP/mouse-AP) IHC kit has been developed by directly labeling anti-mouse immunoglobulins with HRP and AP enzymes using a proprietary tandem hyperlabelling technology. This ensures consistent and reproducible immunodetection of 2 mouse monoclonal antibodies. Nuclear, cytoplasmic, and membrane antigens in different types of tissues can be readily detected.

The MULTIVIEW® (mouse-HRP/mouse-AP) IHC kit allows the user to detect two different mouse antibodies on the same section via sequential staining and chromogen development. The reagents can be used for manual staining or with automated staining instruments and are specifically designed for multiplex immunohistochemical staining assays.

KIT CONTENTS

1. Peroxidase Block	10 mL
2. POLYVIEW® IHC reagent (mouse-HRP) (Prod. no. ADI-950-112)	10 mL
3. POLYVIEW® IHC reagent (mouse-AP) (Prod. no. ADI-950-110)	10 mL
4. HIGHDEF® IHC chromogen substrate (DAB, stable) (Prod. no. ADI-950-212) buffer and chromagen	15 mL / 1 mL 15 mL / 0.5 mL
5. HIGHDEF® red IHC chromogen (AP) (Prod. no. ADI-950-140) buffer & chromogen	
6. Empty Mixing Bottles	2 bottles

STORAGE

Store kit and reagents at 2-8°C in the dark. Do not freeze.

STABILITY

12-24 months (see expiration date on reagent bottles).

COMPOSITION

Peroxidase Block, The POLYVIEW® detection reagents, Mouse HRP and Mouse AP, are provided in ready to use format. HIGHDEF® IHC chromogen substrate (DAB, stable), and HIGHDEF® red IHC chromogen (AP) solutions solutions require preparation before use.

Refer to “Preparation” sections for this information.

OTHER MATERIALS NEEDED

- Xylene or dewaxing reagents
- Absolute ethanol
- Distilled or deionized water
- IHC wash buffer (Prod. no. ADI-950-235)
- Pre-Blocking solution: IHC background blocker (Prod. no. ADI-950-230, optional)
- IHC diluent (primary Ab) (Prod. no. ADI-950-244)
- Counterstain
- Mounting Medium: HIGHDEF® mount (Prod. no. ADI-950-261)

PRECAUTIONS

1. Wear appropriate personal protective apparel. Avoid contact with clothes and exposed skin. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
2. Interpretation of the results is the sole responsibility of the user.

RECOMMENDED STAINING PROTOCOL

1. Paraffin-embedded tissue sections must be deparaffinized with xylene or dewaxing agent and rehydrated with a graded series of ethanol and water washes before staining. Follow the standard dewaxing and rehydration protocol used in your lab.
2. The investigator needs to optimize the dilution and incubation times for primary antibodies.
3. Each immunostaining run should include known positive and negative controls to assure proper functioning of the staining system and aid in valid interpretation of the results.

Typical controls:

Positive Control: A tissue known to contain the desired antigen, which has yielded positive staining in the past.

Negative Controls:

Reagent Controls

A. Substitute normal non-immune serum from the same host animal as the primary antibody (e.g. if using mouse monoclonal primary antibodies, use mouse non-immune serum).
B. Substitute matching host species isotype control for primary antibody
C. Use antigen-adsorbed primary antibody (i.e. antibody reagent which has been adsorbed with the target antigen to remove specific antibody)

Tissue control – A tissue known to not contain the desired antigen.

4. Consult the primary antibody supplier for recommended antigen recovery treatments. Perform epitope recovery pretreatments before starting staining procedures.
5. Once the slide treatment has been started, DO NOT let tissues or specimens dry. This can cause undesirable background or artifacts.

Preparation of HIGHDEF® IHC chromogen substrate (DAB, stable) Working Solution

1. Transfer 1mL of HIGHDEF® IHC chromogen substrate (DAB, stable) buffer to a tube or mixing bottle.
2. Add 1 drop (approximately 20 µL) of HIGHDEF® IHC chromogen substrate (DAB, stable) chromogen to the buffer and mix thoroughly.
3. HIGHDEF® IHC chromogen substrate (DAB, stable) working solution is stable for 2 weeks refrigerated at 2-8°C.
4. Working solution volume can be scaled up using the same ratio of buffer to chromogen.
5. Dispose of unused HIGHDEF® IHC chromogen substrate (DAB, stable) working solution in appropriate waste stream according to local, state or federal regulations.

Preparation of HIGHDEF® red IHC chromogen (AP) Substrate Working Solution

1. Transfer 1 mL of HIGHDEF® red IHC chromogen (AP) Buffer to a tube or mixing bottle.
2. Add 1 drop (approximately 20 µL) of HIGHDEF® red IHC chromogen (AP) chromogen to buffer and mix thoroughly.
3. HIGHDEF® red IHC chromogen (AP) substrate working solution should be used within 20-30 minutes of preparation. Discard any solution not used within this period.
4. Working solution volume can be scaled up using the same ratio of buffer to chromogen.
5. Dispose of unused HIGHDEF® red IHC chromogen (AP) substrate working solution in appropriate waste stream according to local, state, and federal regulations.

STAINING PROCEDURE

		INCUBATION TIME
1. Perform antigen retrieval (if necessary)	Recommended solutions: IHC enzyme antigen retrieval reagent (Prod. No. ADI-950-280), Antigen Retrieval Reagent, pH 6 (10X) (Prod. No. ENZ-ACC112), or Antigen Retrieval Reagent, pH 9 (10X) (Prod. No. ENZ-ACC113).	**5-25 min.**
2. Block endogenous HRP activity	Incubate with Peroxidase Block Wash slides with 3 changes of IHC wash buffer.	**5– 10 min.** **3 x 1 min.**
3. Apply first primary mouse antibody	Apply mouse primary antibody that will be used with HRP. Wash slides with 3 changes of IHC wash buffer.	**30 min.** **3 x 1 min.**
4. Apply Mouse HRP Solution	Apply POLYVIEW® IHC reagent (Mouse HRP) solution. Wash slides with 3 changes of IHC wash buffer.	**20 min.** **3 x 1 min.**
5. Develop with HIGHDEF® substrate (DAB, stable)	Apply the HIGHDEF® IHC chromogen substrate (DAB, stable) solution and incubate. Wash slides with 3 changes of IHC wash buffer.	**5 min.** **3 x 1 min.**

6. Apply second primary mouse antibody	Apply the second mouse primary antibody that will be used with AP. Wash slides with 3 changes of IHC wash buffer.	**30 min.** **3 x 1 min.**
7. Apply Mouse AP Solution	Apply POLYVIEW® IHC reagent (Mouse-AP) solution. **Wash slides with 3 changes of IHC wash buffer.**	**20 min.** **3 x 1 min.**
8. Develop with HIGHDEF® Red (AP)	Apply the HIGHDEF® red IHC chromogen (AP) solution and incubate. **Wash slides with 3 changes of IHC wash buffer.**	**15 min.** **3 x 1 min.**

9. Counterstain	Incubate tissue with Counterstain (e.g. Hematoxylin), according to manufacturer's recommendation or standard laboratory protocol. **Wash slides with water 3 times, followed by 1 time in IHC wash buffer, then 1 time in water.**	**1-5 min.** **5 x 1 min.**
10. Dehydrate & Coverslip	Dehydrate tissues through graded ethanol series, followed by xylene series. Apply coverslips with permanent mounting medium.	

NOTE: The use of an antibody removal buffer between the first layer and the second is optional. Although, sufficient washing, as indicated in the staining protocol (3 x 1 minute washing with IHC wash buffer), will remove excess antibody, detection reagent, or chromogen making the staining of each antigen of interest specific.

NOTES

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