Product Manual

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

Catalog #: ADI-950-100

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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.

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INTENDED USE

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

The MULTIVIEW®(mouse-HRP/rabbit-AP) IHC kit has been developed by directly labeling anti-rabbit and anti-mouse immunoglobulins with enzymes using a proprietary tandem hyperlabelling technology. This ensures consistent and reproducible immunodetection of mouse and rabbit antibodies with a single reagent. Nuclear, cytoplasmic, and membrane antigens in different types of tissues can be readily detected. The single step MULTIVIEW®(mouse-HRP/rabbit-AP) IHC kit enables faster staining procedures than traditional two-step methods using biotin and avidin/streptavidin conjugates by being able to directly label mouse antibodies with HRP and rabbit antibodies with AP at the same time, all with significantly lower background staining.

The MULTIVIEW®(mouse-HRP/rabbit-AP) IHC kit is suitable for use with mouse and rabbit antibodies, both monoclonal and polyclonal. The reagents can be used for manual staining or with automated staining instruments and is 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)	5 mL
3. POLYVIEW® IHC reagent (rabbit-AP) (Prod. no. ADI-950-111)	5 mL
4. HIGHDEF® IHC chromogen substrate (DAB, stable) (Prod. no. ADI-950-212) buffer and chromogen	15 mL/1 mL
5. HIGHDEF® red IHC chromogen (AP) (Prod. no. ADI-950-140) buffer & chromogen	15 mL/0.5 mL
6. Empty Mixing Bottles	2 bottles

STORAGE

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

STABILITY

12 months (see expiration date on reagent bottles).

COMPOSITION

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

Refer to “Preparation” and “Protocol” sections for this information.

OTHER MATERIALS NEEDED

- Xylene or dewaxing reagents
- Absolute ethanol
- Distilled or de-ionized water
- Immunostaining wash buffer (Prod. No. ADI-950-235)
- IHC background blocker (Prod. No. ADI-950-230, optional)
- IHC diluent (primary Ab) (Prod. No. ADI-950-244)
- Counterstain
- HIGHDEF® mount (Prod. No. ADI-950-261)
- Mixing tubes for Multiplex Secondary Solution Reagents A and B

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 for antigen recovery treatments. Perform epitope recovery pretreatments before starting the staining procedure.
5. Once the slide treatment has been started, DO NOT let tissues or specimens dry. This can cause undesirable background or artefacts.

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

1. Transfer 1 mL of the HIGHDEF® IHC chromogen substrate (DAB, stable) buffer to a tube or mixing bottle.
2. Add 1 drop (approximately 20 µL) of HIGHDEF® substrate (DAB, stable) chromogen to the buffer and mix thoroughly.
3. HIGHDEF® 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® substrate (DAB, stable) working solution in appropriate waste stream according to local, state, and federal regulations.

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

1. Transfer 1 mL of the 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 the buffer and mix.
3. HIGHDEF® IHC red chromogen r (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**	Apply Peroxidase Block	**5– 10 min.**
		Wash slides with 3 changes of IHC wash buffer.	**3 x 1 min**.
3.	**Incubate slides with primary antibodies**	Apply mixed primary antibody set (contains both mouse and rabbit primary antibodies) and incubate.	**30 min.**
		Wash slides with 3 changes of IHC wash buffer.	**3 x 1 min.**
4.	**Mix equal volumes of Mouse HRP and Rabbit AP. Apply for 20 min.**	In a separate tube (not provided) mix equal volumes of POLYVIEW® IHC reagent (mouse-HRP) and POLYVIEW® IHC reagent (rabbit-AP). (Example given: 1 mL of Mouse HRP and 1 mL of Rabbit AP)	**20 min.**
		(Note: Mixed secondary solution is stable only for 24-48hrs.)	
		Apply to slides and incubate at room temperature for 20 min.	**3 x 1 min.**
		Wash slides with 3 changes of IHC wash buffer.	

5.	**Develop HRP-mouse antibody with HIGHDEF® substrate (DAB, stable)**	Apply HIGHDEF® substrate (DAB, stable) solution and incubate.	**5 min.**
		Wash slides with 3 changes of IHC wash buffer.	**3 x 1 min.**
6.	**Develop AP-rabbit antibody with HIGHDEF® red (AP)**	Apply HIGHDEF® red (AP) solution and incubate.	**15 min.**
		Wash slides with 3 changes of IHC wash buffer.	**3 x 1 min.**
7.	**Counterstain**	Apply Counterstain (e.g. Hematoxylin), according to manufacturer's recommendations or standard laboratory protocol.	**1-5 min.**
		Wash slides with water 3 times, followed by 1 time in IHC wash buffer, then 1 time in water.	**5 x 1 min.**
8.	**Dehydrate & Coverslip**	Dehydrate tissues through graded ethanol series, followed by xylene series.	
		Apply coverslips with permanent mounting medium.	

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