Product Manual

POLYVIEW® (mouse on mouse-HRP) IHC kit

Catalog #: ADI-950-114

(For Mouse Primary antibodies)

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Product Manual

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Please read entire booklet before proceeding with the assay.

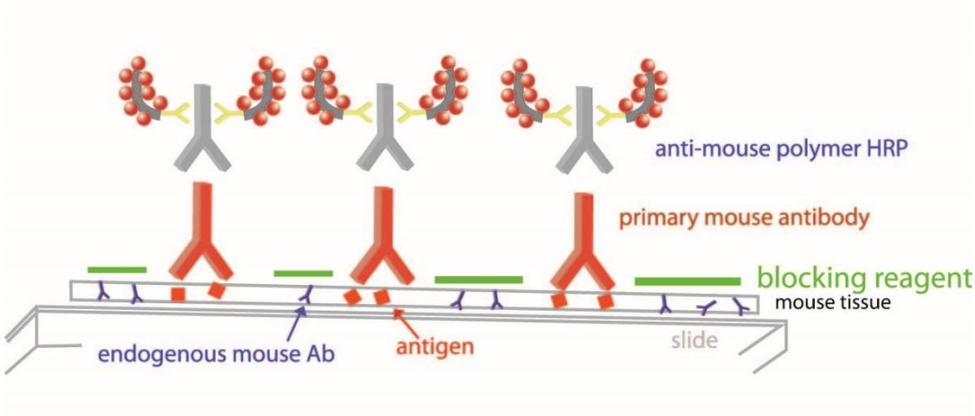
Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTENDED USE

The POLYVIEW® (mouse on mouse-HRP) IHC kit is a non-biotin, one-step detection system suitable for detecting mouse antigens on mouse tissue sections. The detection system may be used with formalin-fixed paraffin embedded and cryostat sections, as well as blood smears, cytosmears, and cell preparations. The POLY-VIEW® IHC kit has been developed by directly labeling anti-mouse immunoglobulins with enzymes using a proprietary tandem hyperlabelling technology. This ensures consistent and reproducible immunodetection of mouse antibodies against nuclear, cytoplasmic and membrane antigens in different types of tissues. The single step system enables faster staining procedures than traditional two-step methods using biotin and avidin/streptavidin conjugates, with significantly lower background.

The POLYVIEW® (mouse on mouse-HRP) IHC kit is suitable for use with mouse IgG and IgM antibodies, both monoclonal and polyclonal. The reagents can be used for manual staining or with automated staining instruments and are well suited for multiplex immunohistochemical staining assays.



REAGENTS SUPPLIED

- IHC tissue primer
- Blocking reagent
- Anti-Mouse HRP Polymer
- DAB Buffer, 15 mL,
- DAB Chromogen 1 mL,
- Empty mixing bottle for DAB

STORAGE

Store at 2°-8°C. Do not freeze.

STABILITY

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

FORMAT

All reagent components are formulated without azide or thimerosol preservatives. The reagents are provided in ready-to-use format with the exception of DAB.

OTHER MATERIALS NEEDED

1. Xylene or dewaxing reagents
2. Absolute ethanol
3. Distilled or deionized water
4. IHC wash buffer (Prod. no. ADI-950-235)
5. IHC diluent (primary Ab) (Prod. no. ADI-950-244)
6. Counterstain
7. HIGHDEF® IHC mount (Prod. no. ADI-950-261)

PRECAUTIONS

1. DAB has been classified as a suspected carcinogen and can cause skin irritation upon contact. 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.

REAGENT PREPARATION

Preparation of DAB working solution.

1. Transfer 1 mL of the DAB Buffer to a tube or mixing bottle.
2. Add 1 drop (approximately 20 µL) of DAB Chromogen to the buffer. Mix thoroughly.
3. The substrate working solution is stable for 1 week 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 DAB solutions in appropriate waste stream according to local, state, or federal regulations.

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 artifacts.

STEP BY STEP PROTOCOL

STEP	STAINING PROCEDURE:	INCUBATION TIME
1. Tissue Primer	A. Incubate slides at room temperature in Tissue Primer. B. Rinse slides with Wash Buffer three (3) times, for 1 min. each time.	5 min. 3 x 1 min.
2. Blocking Reagent	A. Incubate slides at room temperature with Blocking Reagent. B. Drain or blot off solution. Do not rinse.	20 min.
3. Primary Mouse Antibody	A. Incubate with Primary Antibody, prepared according to the manufacturer's recommended protocol at the desired concentration. Concentrated Primary Antibodies may be diluted using Primary Antibody Diluent. B. Wash slides with 3 changes of Immuno Wash Buffer.	30 – 60 min. 3 x 1 min.
4. Mouse HRP Polymer	A. Incubate the tissue with HRP Polymer reagent. B. Wash slides with 3 changes of Immuno Wash Buffer.	20 min. 3 x 1 min.
5. DAB	A. Prepare the DAB substrate working solution (see above). B. Incubate tissue with prepared DAB substrate solution. Monitor level of staining to determine optimal time of incubation. C. Rinse slides with 3 changes of water.	5 – 10 min. 3 x 1 min.
6. Counterstain	A. Incubate tissue with Counterstain (e.g. Hematoxylin), according to manufacturer's recommendation or standard laboratory protocol. B. Wash slides with water 3 times, followed by 1 time in Immuno Wash Buffer, then 1 time in water.	~1 min. 3 x 1 min. H2O 1 x 1 min Buffer 1 x 1 min H2O
7. Dehydrate & Coverslip	A. Dehydrate tissues through graded ethanol series, followed by xylene series. B. Apply coverslips with permanent mounting medium.	

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com