Diagnostic BioSystems

IHC Made Affordable

Mouse/Rabbit UnoVue™ HRP/DAB Detection System

Catalog No. UMR 100PD, UMR 1000PD
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Intended Use: For In Vitro Diagnostic Use

The Mouse/Rabbit UnoVue HRP/DAB Detection System is a non-biotin, one-step detection system suitable for demonstrating antigens in formalin-fixed paraffin-embedded tissues and cryostat sections. The UnoVue Detection System may also be used with blood smears, cytosmears, and cell preparations.

The UnoVue detector kits have been developed by directly labeling anti-mouse and anti-rabbit 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 detected readily. The single step UnoVue Detection System enables faster staining procedures than traditional two-step methods using biotin and avidin/streptavidin conjugates, with significantly lower background.

The Mouse/Rabbit UnoVue HRP/DAB Detection System is suitable for use with mouse and rabbit IgG and IgM antibodies, both monoclonal and polyclonal. The reagents can be used for manual staining or with automated staining platforms.

Kit Contents Sufficient reagents are provided for 100 tests

1. Peroxidase Block	10 mL	100 mL
2. Anti-Mouse/Rabbit HRP Polymer	10 mL	100 mL
3. Stable DA1B/Plus Buffer	15 mL	200 mL
4. Stable DAB/Plus Chromogen	1 mL	5 mL
5. Empty mixing bottle for Stable DAB/Plus	15 mL bottle	15 mL bottle

Storage and Handling Store at 2°-8°C away from light. Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

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

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

Material Required But Not Provided Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com.

Preparation of Stable DAB/Plus Substrate Working Solution

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

Precautions

i) 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.
ii) Interpretation of the results is the sole responsibility of the user.

Troubleshooting If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is

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suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com

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	STAINING PROCEDURE:	INCUBATION TIME
1. Peroxidase Block	A. Incubate slides in Peroxidase Block. B. Rinse slides with Immuno Wash Buffer three (3) times, for 1 min. each time.	**5 min.** **3 x 1 min.**
2. Pre-Blocking (optional)	A. Add 2 drops (100 µL) or enough volume of Pre-Blocking Solution to cover the tissue section. B. Drain or blot off solution. Do not rinse.	**10 min.**
3. Primary Mouse or Rabbit 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. UnoVue Mouse/ Rabbit HRP Polymer	A. Incubate the tissue with UnoVue HRP Polymer reagent. B. Wash slides with 3 changes of Immuno Wash Buffer.	**15 min.** **3 x 1 min.**
5. Stable DAB/Plus	A. Prepare the Stable DAB/Plus substrate working solution (see above). B. Incubate tissue with prepared Stable DAB/Plus 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. H_2O** **1 x 1 min Buffer** **1 x 1 min H_2O**
7. Dehydrate & Coverslip	A. Dehydrate tissues through graded ethanol series, followed by xylene series. B. Apply coverslips with permanent mounting medium.	

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