Diagnostic BioSystems

IHC Made Affordable

Mouse/Rabbit PolyVue Plus™ HRP/DAB Detection System

Catalog No: PVP 25D, PVP 100D, PVP 1000D
Document #: DS-6020-A
Effective Date: 05/01/2015

Intended Use

The Mouse/Rabbit PolyVue Plus™ HRP/DAB Detection System is a non-biotin, two-step detection system suitable for labeling antigens in formalin-fixed paraffin-embedded tissues and cryostat sections. The PolyVue Plus™ Detection System may also be used with blood smears, cytosmears, and cell preparations. This System has been developed by directly labeling immunoglobulins with enzymes using a proprietary tandem hyperlabelling technology. This ensures consistent and reproducible immunostaining for all types of nuclear, cytoplasmic and membrane antigens in different types of tissues with significantly lower background than detection systems using biotin and avidin conjugates.

The Mouse/Rabbit PolyVue Plus™ HRP/DAB Detection System can simultaneously detect both mouse and rabbit antibodies. This System is suitable for use with mouse IgG, IgM and rabbit primary antibodies, both monoclonal and polyclonal. This Detection System can be used for manual or automated staining. The increased sensitivity of the Mouse/Rabbit PolyVue Plus™ HRP/DAB Detection System enables faster staining procedures without compromising results. The user may need to further dilute primary antibody due to the superior sensitivity of the PolyVue Plus™ Detection System.

Kit Contents

Reagent Volumes	**25 Tests**	**100 Tests**	**1000 Tests**
Catalog Number	PVP 25D	PVP 100D	PVP 1000D
Tissue Primer™	2.5 mL	10 mL	100 mL
Background Blocker	2.5 mL	10 mL	100 mL
PolyVue Plus™ Mouse/Rabbit Enhancer	2.5 mL	10 mL	100 mL
PolyVue Plus™ Mouse/Rabbit HRP Label	2.5 mL	10 mL	100 mL
Stable DAB/Plus™ Buffer	15 mL	15 mL	200 mL
Stable DAB/Plus™ Chromogen	1 mL	1 mL	5 mL
Empty mixing bottle for Stable DAB/Plus	3 mL	15 mL	15 mL

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Storage

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)

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

Composition

All reagent components are formulated without azide or thimerosol preservatives. The reagents are provided in ready-to-use form, 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 40 µL) of Stable DAB/Plus Chromogen to the buffer.
3. Mix thoroughly.
4. The substrate working solution is stable for two weeks refrigerated at 2-8°C.
5. Working solution volume can be scaled up using the same ratio of buffer to chromogen.
6. Dispose of unused Stable DAB/Plus Substrate working solution in appropriate waste stream according to local, state, and 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 as per routine histology methods.
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.
4. Consult the primary antibody supplier for recommended for antigen recovery pre-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.

Typical Controls

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

B. Negative Controls: Reagent Controls
   - 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).
   - Substitute matching host species isotype control for primary antibody
   - Use antigen-adsorbed primary antibody (i.e. antibody reagent which has been adsorbed with the target antigen to remove specific antibody)

C. Negative Controls: Tissue control – A tissue known to *not* contain the desired antigen.

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Staining Protocol Continued

Step	Instruction	Wash/Time
Pretreatment	Refer primary antibody data sheet.	3x2min
Tissue Primer™	Apply reagent and incubate for 5 min at room temperature.	3x2min
Background Blocker	Apply reagent and Incubate for 5 min at room temperature.	Do Not wash, only blow
Primary Antibody	Refer antibody data sheet.	3x2min
PolyVue Plus™ Enhancer	Apply reagent and incubate for 10 min at room temperature.	3x2min
PolyVue Plus™ HRP	Apply reagent and incubate for 10 min at room temperature.	3x2min
DAB/Plus™	Apply reagent and incubate for 5 min at room temperature.	3x2min
Mayer's Hematoxylin	Apply reagent and incubate for 2 min at room temperature.	3x2min
Dehydration/Clearing/Mounting	Follow routine laboratory methods.	N/A

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