Ultra High Def™ Polymer Mouse HRP Kit

Catalog No: P1-M-100-HRP-Kit, P1-M-Auto-100-HRP Kit
Document No: IFU_P1_M_100_HRP-Kit
Effective Date 12/04/2017, IFU-167 Rev A

Intended Use

For In Vitro Diagnostic Use

The Ultra High Def ™ Mouse HRP Kit is a non-biotin, one-step detection system suitable for detecting antigens in formalin-fixed paraffin-embedded tissues and cryostat sections, as well as blood smears, cytosmears, and cell preparations.

The Ultra High Def™ HRP detection kits have 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 Mouse HRP Polymer Detection System enables faster staining procedures than traditional two-step methods using biotin and avidin/streptavidin conjugates, with significantly lower background.

The Ultra High Def™ Mouse HRP Detection System 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.

Introduction

Optimal immunostaining not only depends on the specificity of the primary antibody and other immunoreagents but also depends on obtaining a good signal to noise ratio. Binding of an antibody to its epitopes involves van der Waals forces, electrostatic forces and hydrophobic forces. Certain antibodies have tendency to bind loosely and nonspecifically to unrelated epitopes, which can create undesired background staining. In order to remove these nonspecifically bound antibodies, a thorough washing is required after each immunostaining step. Immuno Wash Buffer is specifically designed to remove such loosely bound antibodies effectively and efficiently and to provide a cleaner background staining.

Kit Contents

1. Ultra High Def™ Peroxidase/AP Block	100 mL
2. Ultra High Def™ Background Blocker	100 mL
3. Ultra High Def™ Polymer Mouse-HRP	100 mL
3. Ultra High Def™ DAB Buffer	200 mL
4. Ultra High Def™ DAB Chromogen	5 mL
5. Ultra High Def™ Hematoxylin	100 mL

Storage

Store at 2 - 8°C. Do not freeze. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Stability is 12-24 months (see expiration date on reagent bottles)

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Composition

All reagent components are formulated without azide or thimerosol preservatives. The reagents are provided in ready-to-use form, with the exception of Ultra High Def™ DAB Chromogen. SDS is available upon request.

Material Required But Not Provided

1. Xylene or dewaxing reagents
2. Absolute ethanol
3. Distilled or deionized water
4. Immuno Wash Buffer
5. Primary Antibody Diluent (if required)
6. Coverslips and mounting media

Preparation of Stable DAB/Plus Substrate Working Solution

1. Transfer 1 mL of the Ultra High Def™ DAB Buffer to a tube or mixing bottle.
2. Add 1 drop (approximately 20 µL) of Ultra High Def™ DAB Chromogen to the buffer. Mix thoroughly.
3. The substrate working solution is stable for 24 hours 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.

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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.
iii) Consult local and/or state authorities with regard to recommended method of disposal
iv) Microbial contamination could produce erroneous results
v) Do not ingest or inhale any reagents. Reagents may cause irritation, avoid contact with eyes and mucous membranes. If reagents contact these areas, rinse with copious amounts of water.

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)
Negative 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.

	MANUAL STAINING PROCEDURE	**INCUBATION TIME**
1. Perform antigen retrieval (if necessary)	.Recommended solutions: Universal Enzyme , 10x EDTA Retrieval Buffer, pH 8.0 (ACR-031), or 10X Citrate Buffer, pH 6.0 (ACR-002)	Refer to Antibody Data Sheet for recommendations
2. Peroxidase Block	A. Incubate slides in peroxidase block.	10 minutes
	B. Rinse slides with Immuno Wash Buffer three (3) times, for 1 min. each time	3 x 1 minute
3. Blocking (optional)	A. Add 2 drops (100 µl) or enough volume of Background Blocker	5 min.
	B. Drain or blot off solution. Do not rinse.	
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.	30 – 60 min.
	B. Wash slides with 3 changes of Immuno Wash Buffer.	3 x 1 min.
4. Ultra High Def™ Mouse-HRP Polymer	A. Incubate the tissue with Ultra High Def™ Mouse HRP Polymer reagent.	15 min.
	B. Wash slides with 3 changes of Immuno Wash Buffer.	3 x 1 min.
5. Ultra High Def™ Stable DAB/Plus	A. Prepare the Stable Ultra High Def™ DAB/substrate working solution (see above).	
	B. Incubate tissue with prepared DAB/substrate solution. Monitor level of staining to determine optimal time of incubation.	5 – 10 min.
	C. Rinse slides with 3 changes of water.	3 x 1 min.
6. Counterstain	A. Incubate tissue with Counterstain (e.g. Hematoxylin), according to manufacturer's recommendation or standard laboratory protocol.	~1 min .
	B. Wash slides with water 3 times, followed by 1 time in Immuno Wash Buffer, then 1 time in water.	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.	

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact StatLab Medical Products Technical Support at 800-442-3573 or email at ihctech@statlab.com

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