Ultra High Def™ Polymer Mouse/Rabbit AP Kit

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Intended Use For In Vitro Diagnostic Use

The Ultra High Def™ Polymer AP Red System is a non-biotin, Universal one-step Mouse/Rabbit detection system suitable for demonstrating antigens in formalin-fixed paraffin-embedded tissues and cryostat sections.

The Ultra High Def™ Polymer AP detection 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 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™ Polymer AP detection system is suitable for use with mouse or rabbit IgG and IgM antibodies, both monoclonal and polyclonal. The reagents have been optimized to be used manual 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.

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.

Kit Contents

Component	P1-U-20-AUTO-AP	P1-U-10-AP
Ultra High Def ™ Peroxidase/AP Block	2 x 10 mL	N/A
Ultra High Def ™ Background Blocker	2 x 10 mL	N/A
Ultra High Def™ Polymer AP (anti-mouse/rabbit)	2x 10 mL	10 mL
Ultra High Def ™ Red-AP Buffer	3 x 10 mL	10 mL
Ultra High Def ™ Red-AP Chromogen	3 x 10 mL	10 mL
Ultra High Def ™ Hematoxylin	2 x 10 mL	N/A

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 form, with the exception of Ultra High Def™ Red AP Chromogen and Buffer. 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 Red AP Substrate Working Solution

1. Transfer equal amounts of the Ultra High Def™ Red AP Buffer and Ultra High Def™ Red AP Chromogen to a tube or mixing bottle. Mix thoroughly.
2. ***MAKE FRESH: Working Dilution is only Stable for 20-30 minutes***.
3. Dispose of unused Ultra High Def™ Red Chromogen and Buffer solutions in appropriate waste stream, according to local, state or federal regulations.

Precautions

Specimens, before and after fixation and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The SDS is available upon request

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.	Do not rinse slides
4. Primary Mouse/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.	Refer to antibody data sheet
	B. Wash slides with 3 changes of Immuno Wash Buffer.	3 x 1 min.
6. Ultra High Def™ Mouse/Rabbit-AP Polymer	A. Incubate the tissue with Ultra High Def™ AP Polymer reagent.	10 min.
	B. Wash slides with 3 changes of Immuno Wash Buffer.	3 x 1 min.
7. Ultra High Def™ Red-AP Chromogen	A. Prepare the Stable Ultra High Def™ Red-AP substrate working solution (see above).	
	B. Incubate tissue with prepared Red-AP 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. ~1 min
8. 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.	3 x 1 min. H_2O
9. Dehydrate & Coverslip	A. Dry slides in 60°C Oven for 10 minutes or air dry for 15 minutes.	
	B. Dip slide quickly in xylene and apply permanent mounting media and Apply coverslip.	
	Note: Aqueous Mounting media may also be used	

AUTOMATED IHC STAINING PROCEDURE (refer to your instrument manual)

PROTOCOL RECOMMENDATIONS:

1. Pretreatment Solution/Protocol: Please refer to the respective primary antibody datasheet for recommended pretreatment solution and

protocol.

2. Peroxidase Block: Block for 10 minutes with Ultra High Def™ Peroxidase/AP block. Rinse with Immuno Wash Buffer
3. Background Block: Incubate for 5 minutes with Ultra High Def™ Background Block. Do not rinse
4. Primary Antibody: Please refer to the respective primary antibody datasheet for recommended primary antibody. Rinse with Immuno Wash Buffer

5. Ultra High Def™ polymer AP: Incubate the tissue with Ultra High Def™ polymer AP reagent for 20 minutes. Rinse with Immuno Wash Buffer
6. Substrate Chromogen Batch: Incubate tissue with freshly prepared Ultra High Def™ Red working solution for between 5 to 15 minutes. Rinse with Distilled water
7. Counterstain: Counterstain with Ultra High Def™ Hematoxylin for 1 minute
8. Allow sections to air dry completely and coverslip with synthetic resin-Slides may be quickly dipped into fresh xylene or xylene substitute if needed to coverslip

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