StatLab Quantum HD 3 Step Polymer HRP Kit (Mouse/Rabbit)

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Intended Use

For In Vitro Diagnostic Use

The StatLab Quantum HDx 3 step Polymer HRP Kit is a non-biotin, three-step detection system suitable for detecting antigens in formalin-fixed paraffin-embedded tissues and cryostat sections. It may also be used with blood smears, cytosmears, and cell preparations. In combination with the StatLab Quantum HD™ IHC instrument, the QHD 3 Step Polymer HRP kit may also be used for chromogenic in situ hybridization (CISH).

These products are used in an IHC or CISH procedure, which allows the qualitative identification by light microscopy of antigens or gene deletions, translocations and amplifications in sections of formalin-fixed, paraffin embedded tissues, via sequential steps with interposed washing steps. The Quantum HD 3 Step Polymer HRP kit can be used for manual immunohistochemistry as well as on any open platform.

On the StatLab Quantum HDx Slide Stainer, paraffin will be removed from the formalin-fixed, paraffin embedded tissues. For IHC, if required by the primary antibody, sections are subjected to epitope retrieval prior to staining. The section is subsequently incubated with optimally diluted primary antibody. For CISH, sections are subjected to enzyme digestion, denaturization and hybridization with the ISH probe. The section is subsequently incubated with ISH antibody to detect the probe.

The Quantum HD HRP polymer is a three-step detection system consisting of HRP Polymer A, HRP Polymer B and HRP Polymer C. It recognizes mouse and rabbit immunoglobulins, and it detects any tissue-bound primary antibody. Sections are further incubated with the substrate/chromogen, 3,3' -diaminobenzidine (DAB) causing a reaction with the peroxidase producing a visible brown precipitate at the antigen site. Sections are counterstained with Hematoxylin. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with an antigen.

Introduction

IHC (Immunohistochemistry) is a commonly used technique supporting the identification of antigens present on the tissues or cells.

CISH (Chromogenic in situ hybridization) is a commonly used technique supporting the identification of gene deletions, Translocations and amplification in cells.

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. Quantum HD Immuno Wash Buffer 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.

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IVD

Kit Contents

Component	QHD-U3-15-HRP-Kit
Quantum HD Dewax Solution 1	2-15 mL
Quantum HD Peroxidase Block	15 mL
Quantum HD HRP-Polymer A	15 mL
Quantum HD HRP Polymer B	15mL
Quantum HD HRP Polymer C	15 mL
Quantum HD DAB Substrate (2x)	7.5 mL
Quantum HD DAB Chromogen (2x)	7.5 mL
Quantum HD Hematoxylin	15 mL

Composition

All reagent components are formulated without azide or thimerosal preservatives. The reagents are provided in ready-to-use form, except for Quantum HD 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. QHD Immuno Wash Buffer -QHD-015
5. Primary Antibody Diluent (if required)
6. Coverslips and mounting media

Preparation of Stable DAB/Plus Substrate Working Solution

1. Transfer 1 part of Quantum HD DAB Substrate (2x) to a tube or mixing bottle.
2. Add 1 part of Quantum HD DAB Chromogen (2x) to the 1 part of Quantum HD DAB (2x) Substrate.
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.

Precautions

1. This product contains Sodium Azide (NaN3). At product concentrations Sodium Azide has not been categorized as hazardous. Sodium Azide may react with lead or copper plumbing to form potentially explosive metal azides. Upon disposal flush with large amounts of water to prevent build-up of metal azides in plumbing.
2. 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.
3. 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. [1]
4. Consult local and/or state authorities regarding recommended method of disposal
5. Microbial contamination could produce erroneous results
6. 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. Wear disposable gloves when handling reagents.
7. There is no expressed or implied warranties which extend beyond this datasheet. StatLab is not liable for personal injury, property damage or economic loss caused by this product.

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.

AUTOMATED IHC STAINING PROCEDURE (refer to your instrument manual)

PROTOCOL RECOMMENDATIONS:

1. The slides should be baked to remove any residual water, additional baking/drying time may be included in the IHC slide protocol.
2. D is removed using the Quantum HD Dewax Solution 1 (DS1-50). Rinse with Quantum HD Wash Buffer.
3. Quantum HD Dewax Solution 2 (DS2-50) may be used to enhance the residue removal and signal (optional and antibody dependent-refer to antibody specification sheets for recommendations for use). Rinse with Quantum HD Wash Buffer.
4. Peroxidase Block: Optional. Rinse with Quantum HD Wash Buffer.
5. Pretreatment Solution/Protocol: Please refer to the respective primary antibody datasheet for recommended pretreatment solution and protocol.
    a. Heat treatment is performed on the StatLab Quantum HD IHC instrument using Quantum HD retrieval solutions-TR1 or TR2 (offered separately)
    b. Rinse with Quantum HD Wash Buffer.
6. Background Block: Optional. If used, do not rinse with Quantum HD Wash Buffer.
7. Primary Antibody: Please refer to the respective primary antibody datasheet for recommended primary antibody. Rinse with Quantum HD Wash Buffer
8. Apply Quantum HD HRP-Polymer A (enhancer localizes mouse and rabbit antibodies) for recommended minutes. Rinse with Quantum HD Wash Buffer.
9. Apply Quantum HD HRP Polymer-B (reacts with HRP-A) for recommended minutes. Rinse with Quantum HD Wash Buffer.
10. Apply Quantum HD HRP Polymer-C (reacts with HRP-B) for recommended minutes. Rinse with Quantum HD Wash Buffer.
11. Substrate Chromogen On-Board Mixing: Incubate tissue with freshly prepared Quantum HD DAB and Substrate will be accomplished by on-board mixing of the QHD HRP Buffer and QHD HRP Chromogen (1:1) working solution for recommended time. Rinse with Distilled water.
12. Counterstain: Counterstain with Quantum HD Hematoxylin for recommended time. Rinse with Distilled water.
13. Dehydrate through alcohol to xylene, coverslip with synthetic resin.

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

References

Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Supplements 61(01):1-101, 2012.

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