StatLab Quantum HDx™ 2 Step APx Polymer Kit

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Intended Use For In Vitro Diagnostic Use

The Quantum HD 2 Step APx Polymer System is an enhanced non-biotin, two-step Mouse/Rabbit detection system suitable for demonstrating antigens in formalin-fixed paraffin-embedded tissues and cryostat sections. The Quantum HD 2 step APx Polymer Kit may also be used with blood smears, cytosmears and cell preparations.

The Quantum HD 2 Step APx Polymer System utilize secondary antibodies labeled with alkaline phosphatase. The Quantum HD 2 Step APx Polymer kit can be used for manual immunohistochemistry as well as any automated IHC stainer operating as an open platform.

The Quantum HD APx Polymer is a two-step detection system consisting of polymer enhancer and AP Polymer 2x. It recognizes mouse and rabbit immunoglobulins and it detects any tissue-bound primary antibody. If required by the primary antibody, sections are subjected to epitope retrieval prior to staining. Section are further incubated with the substrate/chromogen, red. Reaction with the alkaline phosphatase produces a visible red precipitate at the antigen site. Sections are counterstained with Hematoxylin. Results are interpreted using a light microscope and aid in the differential diagnosis of physiophysiological processes, which may or may not be associated with a particular antigen

The Quantum HD 2 Step APx Polymer 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. Quantum HD 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	Quantity
Quantum HD Dewax Solution 1	2-15 mL
Quantum HD Polymer Enhancer	1-15 mL
Quantum HD AP Polymer 2x	1-15 mL
Quantum HD Red Substrate (2x)	1-7.5 mL
Quantum HD Red Chromogen (2x)	1-7.5 mL
Quantum HD Hematoxylin	1-15 mL

Composition All reagent components are formulated without azide or thimerosal preservatives. The reagents are provided in ready-to-use form for StatLab Quantum HDx IHC instrument with On-Board mixing of Quantum HD AP Chromogen and Buffer. SDS is available upon request.

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Material Required but Not Provided

1. Xylene or dewaxing reagents
2. Absolute ethanol
3. Distilled or deionized water
4. Quantum HD Wash Buffer (diluted 1 portion of wash buffer to 19 parts of distilled or deionized water)-# QHD-015
5. Primary Antibody Diluent (if required)
6. Coverslips and mounting media

Preparation of Stable Red AP Substrate Working Solution

1. StatLab Quantum HDx instrument is capable of on board mixing of QHD Red Substrate (2x) and QHD Red Chromogen (2x)
2. Dispose of unused Quantum HD Red Chromogen and Buffer solutions in appropriate waste stream, according to local, state or federal regulations. Once mixed, solution is stable for only 20-30 minutes.

Precautions

1. 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].
2. Consult local and/or state authorities regarding recommended method of disposal.
3 Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. Wear disposable personal protective gear including gloves.
4. Microbial contamination of reagents may result in an increase in nonspecific staining.
5. Do not use after expiration date stated on the vial. The user must validate any storage conditions other than those Specified in the package insert.
6. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
7. The SDS is available upon request
8. Specimens before and after fixation and all materials exposed to them, should be handled as if capable of transmitting Infections and disposed of with proper precautions.

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. The Quantum HDx instrument may be used to perform these duties and eliminate the need for manual deparaffinization and rehydration of the slides.
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. Deparaffinization 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 using Quantum HD retrieval solutions-TR1 or TR2 (offered separately)

b. Rinse with Quantum HD Wash Buffer.

6. Background Block: Optional. 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. Quantum HD Polymer Enhancer AP (enhancer localizes mouse antibodies): Incubate the tissue with Quantum HD Polymer Enhancer reagent for recommended minutes. Rinse with Quantum HD Wash Buffer.
9. Quantum APx Polymer-2 (localizes rabbit antibodies): Incubate the tissue with Quantum HD APx Polymer 2 for recommended minutes. Rinse with Quantum HD Wash Buffer.
10. 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 Buffer and QHD Chromogen (1:1) working solution for recommended time. Rinse with Distilled water
11. Counterstain: Counterstain with Quantum HD Hematoxylin for recommended time. Rinse with Distilled water.
12. Allow sections to air dry completely and coverslip with synthetic resin-Slides may be quickly dipped into fresh xylene or xylene substitute if needed for cover slipping.

Interpretation of Results

A qualified Pathologist is entitled to give a clinical interpretation of the results obtained based on the patient's clinical history and complementary morphological tissue observations. It is the responsibility of the user to identify the best working conditions and the best reagents to perform the staining run.

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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