StatLab Quantum HDx Manual 2 Step AP Polymer Kit

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Intended Use For In Vitro Diagnostic Use

The Quantum HD 2 Step AP Polymer System is a 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 AP Polymer Kit may also be used with blood smears, cytosmears and cell preparations.

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

The Quantum HD AP Polymer is a two-step detection system consisting of polymer enhancer and AP Polymer 2. 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 physio physiological processes, which may or may not be associated with a particular antigen

The Quantum HD 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. 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	SAM-QHD-U2-15 AP Kit	QHD-U2-100 AP kit
Quantum HD Dewax Solution 1	N/A	N/A
Quantum HD Polymer Enhancer	1-15 mL	100 mL
Quantum HD AP Polymer 2	1-15mL	100 mL
Quantum HD Red Substrate (2x)	1-15mL	50 mL
Quantum HD Red Chromogen (2x)	1-15mL	50 mL
Quantum HD Hematoxylin	1-15mL	100 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 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

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5. Primary Antibody Diluent (if required)
6. Coverslips and mounting media
7. Heat epitope retrieval solution-see antibody specification sheet

Preparation of Stable Red AP Substrate Working Solution

1. Transfer equal amounts of the Quantum HD Red Substrate (2x) and Quantum HD Chromogen (2x) to a tube or mixing bottle. Mix thoroughly.
2. ***MAKE FRESH: Working Dilution is only Stable for 20-30 minutes***.
3. Dispose of unused Quantum HD Red Chromogen and Buffer solutions in appropriate waste stream, according to local, state or federal regulations.
4. The reagents are provided in ready-to-use form for StatLab Quantum HDx IHC instrument with On-Board mixing (1:1) of Quantum HD AP Chromogen and Buffer

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 Manual Staining Protocol

1. Paraffin embedded tissue sections must be dried for sufficient time to allow the tissue to withstand the rigors of heated pretreatments and IHC procedures. Slides 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.

Controls

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)	Refer to Antibody Specification Sheets for recommended Epitope Retrieval Solution and methods	Refer to Antibody Data Sheet for recommendations
2. 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.
3. Quantum HD Polymer Enhancer	A. Incubate the tissue with Quantum HD Polymer Enhancer.	15 min.
	B. Wash slides with 3 changes of Immuno Wash Buffer.	3 x 1 min.

4. Quantum HD AP Polymer 2	A. Incubate the tissue with Quantum HD AP Polymer 2.	15 min
	B. Wash slides with 3 changes of Immuno Wash Buffer	3 x 1 min.
5. Quantum HD (Red) AP Substrate/Chromogen Mixture	A. Prepare the Quantum HD Red-AP substrate working solution by mixing equal amounts of Red Substrate (2x) to Red Chromogen (2x)	10-15 min.
	B. Incubate tissue with prepared Red-AP substrate solution. Monitor level of staining to determine optimal time of incubation.	3 x 1 min. ~1 min
	C. Rinse slides with 3 changes of water.	
6. Counterstain	A. Incubate tissue with Quantum HD Hematoxylin or other appropriate OR Counterstain according to manufacturer's recommendation or standard laboratory protocol.	1-5 min.
	B. Wash slides with water 3 times.	3 x 1 min. H_2O
7. Dehydrate & Coverslip	A. Air dry slides completely-do not rinse in alcohol, chromogen is not resistant to alcohol. B. Once slides are dry, may dip the slides ***quickly*** in one change of freshly poured xylene for cover slipping.	

8. 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.

9. 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

10. References

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

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