QuatroSol II 10X Tris EDTA Buffer For Heat Induced Epitope Recovery, pH 9.0 kit

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

The Antigen Retrieval technique is a novel method for the recovery of antigens from formalin-fixed, paraffin-embedded tissue. It consists of heating tissue sections in the presence of an antigen retrieval solution. The quality of the staining result is largely dependent on strict adherence to the antigen retrieval protocol. If the antigens are incompletely retrieved, the staining is light and the background may be high.

Features

1. Performs four steps simultaneously
   - deparaffinization
   - rehydration
   - antigen retrieval
   - Blocks endogenous peroxidase and alkaline phosphatase.
2. Eliminates the use of alcohols and xylene in deparaffinization process. Saves money and hazardous disposal.
3. When used in conjunction with mounting medium for mounting slides, the entire process from beginning to end is alcohol and xylene-free, thereby eliminating the use of hazardous chemicals.

Known Applications

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Product Description

10X Tris EDTA Buffer For Heat Induced Epitope Recovery, pH 9.0 is designed for use during the heat induced epitope retrieval (HIER) step prior to immunohistochemistry on formalin-fixed paraffin embedded tissue sections. The use of this buffer in combination with heat (often by microwave, water bath, or pressure cooker) has been shown to restore the antigenicity of proteins modified during the formalin fixation of tissue. This buffer is supplied as a 10X stock solution.

Format

10X Concentrated

Volume/UOM

QuatroSol II 10X Tris EDTA Buffer For Heat Induced Epitope Recovery, pH 9.0 (K079)	500 mL
10X Hot Rinse Buffer (K084)	500 mL

Principles of the Procedure

Formalin fixation of tissues induces protein cross links that help in maintaining the cellular morphology by inactivating the digestive enzymes and preserving the cytoskeleton. Fixation stops tissue autolysis, preserves tissue structures, and immobilizes antigens. However, antigens may undergo alteration of their primary, secondary and tertiary structures during fixation. This may cause a loss of reactivity of a specific antibody to that antigen. High-temperature treatment of such proteins in appropriate pH leads to restoration of the epitope structure and hence retrieves the reactivity of antibody to the target antigen. This process is defined as Antigen Retrieval (Shi et al 1991). It has been suggested that antigen retrieval loosens or breaks the cross linkages induced by formalin. This allows for the enhanced penetration of antibodies and accessibility of epitopes.

Materials Required But Not Provided

Diagnostic BioSystems Montage Opus® Antigen Retrieval System [AR 360]

Storage and Handling

Store at room temperature. Do not use after expiration date printed on label. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly.

Specimen Preparation

Appropriate fixation plays an important role in preserving the tissue structure. The antigen retrieval protocol is recommended for use in tissues that have been fixed in formalin only. Ensure that the fixed sections are adequately embedded in paraffin. Cut tissue sections to 4-5 microns.

Precautions

1. Wear disposable gloves when handling reagents.
2. 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.
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the label.
6. The MSDS is available upon request.
7. Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

Preparation of Working Solutions (1x)

8. The 10X concentrated format should be diluted tenfold with distilled or deionized water.
9. Mix one part of concentrated Antigen Retrieval Solution with nine parts of deionized or distilled water.
10. Prior to dilution, shake the bottle vigorously to completely mix the components of the concentrate (the solution may separate into phases over time).
11. Store with cap tightly secured.

Protocol Recommendations

1. Place QuatroSol II solution into a container
2. Prepare a second bath as Hot Rinse
3. Hot Rinse bath can use wash buffer + 0.1% NP9
4. Place Hot Rinse bath in pressure cooker.
5. Heat both containers to about 60°C

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6. Place slides (with paraffin) into preheated QuatroSol II solution
7. Heat slides in Pressure Cooker under high pressure for 15 minutes.
8. Allow Pressure Cooker to cool to about 90˚C (about 15 minutes).
9. Remove slides from QuatroSol II solution and place in Hot Rinse solution for 5 minutes
10. Agitate slides, remove from Hot Rinse, and tap off excess fluid onto a paper towel. Do not allow slides to dry out.
11. Place slides in a wash buffer bath.
12. Allow slides to equilibrate with buffer bath for about 10 minutes.
13. Proceed with IHC staining.

Quality Control

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011

Troubleshooting

1. No deparaffinization and hydration steps. Put slides straight into QuatroSol I solution.
2. Avoid allowing the solution to boil over as this may cause the tissue to come off the slide or the tissue to dry out.
3. Non-specific staining in the negative control can be due to the exposure of endogenous biotin. Over retrieval can sometimes result in high background due to the detection system or excess antibody. In that case further dilution of the antibody may be necessary. Including an additional avidin-biotin block should prevent staining of the exposed biotin.
4. If positive control gives optimum signal, negative control shows no background and test slide gives negative or weak signal, a fixative other than neutral buffered formalin mayhave been used. In order to obtain the best signal under these circumstances, optimization of antigen retrieval conditions is recommended.
5. Refer to appropriate antibody and detection system inserts for pattern and intensity of staining with different antibodies.
6. Contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2, techsupport@dbiosys.com or your local distributor to report unusual staining.

Limitations of the Procedure

The antigen retrieval protocol is recommended for use with tissues fixed *with formalin only*. Other fixatives or fixation procedures may not produce comparable results. Interpretation of the staining results is solely the responsibility of the user.

Warranty

There are no warranties, expressed or implied, which extend beyond this description. Diagnostic BioSystems is not liable for property damage, personal injury, or economic loss caused by this product.

Expected Results

Antigen retrieval can produce markedly improved staining of a wide variety of monoclonal and polyclonal antibodies. This helps overcome false negative staining of over fixed tissue, expand the range of antibodies that can be used and increase the usefulness of archival tissue.

Optimized antigen retrieval should improve signal to noise in immunohistochemistry.

Performance Characteristics

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Diagnostic BioSystems products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

References

I. Shi et al. J Histochem Cytochem 39: 741, 1991

Patented Technology: US 10,281,374,B2

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