

DP3 for One-Step Deparaffinization

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

The DP3 reagent is used for deparaffinization of paraffin-embedded tissue sections. Deparaffinization is completed in a single step without the use of xylene or alcohol. After deparaffinization the tissue sections can be transferred directly into antigen retrieval solution.

Features

1. Performs the following steps
 - Deparaffinization
Removes paraffin both around and beneath the tissue providing better paraffin removal than similar aqueous deparaffinization solutions.
 - Water miscible reagent so slides can be transferred directly to antigen retrieval solution
 - Does not use xylene or alcohol
 - Reagent is nonflammable and biodegradable

Known Applications

Deparaffinization of paraffin-embedded tissue sections.

Product Description

DP3 is a blend of aqueous buffers and a paraffin solvent (orange oil). Because of these properties it can act as both a deparaffinizing agent and a rehydration agent thus eliminating the requirement for transferring the paraffin tissues through a series of graded xylenes and alcohol. Deparaffinization occurs rapidly at temperatures in excess of 50 C. (Range 50 - 80 C). In contrast to other aqueous deparaffinization reagents, the paraffin is completely removed from both around and beneath the tissues.

DP3 deparaffinization is compatible with Immunohistochemistry.

Format

Ready-To-Use. Do not dilute.

Volume/UOM

500 mL

Principles of the Procedure

Paraffin-embedding of tissues is standard practice for many histological techniques. The paraffin provides a solid matrix to aid in cutting of the tissue and preparing histological sections on microscope slides. Prior to staining the paraffin must be removed from the tissue sections as the paraffin interferes with the staining procedure

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.

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

- Wear disposable gloves when handling reagents.
- 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.
- Do not use reagent after the expiration date printed on the label.
- The MSDS is available upon request.
- Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

Preparation of Working Solutions

- The DP3 reagent is provided Ready-To-Use and should not be diluted.
- Store with cap tightly secured.
- The reagent may be reused up to five times.
- Discard used reagent if slides are incompletely deparaffinized.

Protocol Recommendations

1. Fill a suitable container with DP3 reagent.
2. Preheat the DP3 reagent to 50 – 80 C. If using a pressure cooker, set the pressure cooker to the lowest temperature setting.
3. Place slides, containing paraffinized tissue, into the preheated DP3.
4. Generally the paraffin will be dissolved in about five minutes. However, certain paraffin formulations may require extra time. The time in the preheated DP3 can be extended, as needed, up to 20 minutes.
5. After heating, remove slides from DP3 and allow excess DP3 to drain, but do not allow the slides to dry out.
6. Place deparaffinized slides directly into Antigen Retrieval solution and proceed with the Antigen Retrieval protocol.
7. After Antigen Retrieval allow slides to equilibrate with wash buffer bath for about 10 minutes.
8. 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

Avoid allowing the Antigen Retrieval solution to boil over as this may cause the tissue to come off the slide or the tissue to dry out.

1. 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.
2. 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 may have been used. In order to obtain the best signal under these circumstances, optimization of antigen retrieval conditions is recommended.
3. Refer to appropriate antibody and detection system inserts for pattern and intensity of staining with different antibodies.
4. 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, and 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

- Mekota AM *et al.* Determination of optimal rehydration, fixation and staining methods for histological and immunohistochemical analysis of mummified soft tissues. *Biotech Histochem.* 2005 Jan-Feb;80(1):7-13

