Tris EDTA, pH9.0 (50X)
Epitope Retrieval Buffer

PRODUCT INFORMATION:
PS009 500ml
PS009 100ml

INTENDED USE

For *in vitro diagnostic* use only

Tris EDTA epitope retrieval buffer will unmask antigenic sites from formaldehyde fixation and allow for subsequent immunostaining. Use of this high pH retrieval buffer will enhance IHC staining.

SUMMARY AND EXPLANATION

PathnSitu offers 50X concentrated High pH(9.0) Tris EDTA retrieval solution. It is a colorless, odorless, azide free clear solution. Formalin or other aldehyde fixation forms protein cross-links that mask the antigenic sites in tissue specimens, thereby giving weak or false negative staining for immunohistochemical detection of certain proteins. The Tris-EDTA based solution is designed to break the protein crosslinks, therefore unmask the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, thus enhancing staining intensity of antibodies.

PRINCIPLE OF THE PROCEDURE

Heat Induced Epitope Retrieval(HIER) involves treating the slides in heated solutions of various buffers and pH ranges that are used to break the cross-links before application of the primary antibody. Heat sources can be a microwave oven, oven, water bath, pressure cooker, autoclave, or a vegetable steamer. This technique was developed by Dr. Shan-Rong Shi, Taylor, and colleagues, who discovered that the degree of epitope recovery is a function of a number of factors, including the timing of when heating occurs and the temperatures involved.
The pH of the retrieval solution is also a very important factor to consider. A pH that is too high or too low will alter the final results.

REAGENT PROVIDED

The reagent is supplied as 100ml,500ml formats of Tris EDTA retrieval buffer, 50X concentrated.

STORAGE AND HANDLING

Storage Recommendations: Store at 2-8°C. When stored at the appropriate conditions, the retrieval buffer is stable until expiry. **Do not use the buffer after expiration date provided on the container.**
Preparation of working solution: The 50X solution should be diluted to 1X with DI water before its use. For example: To make 1 litre of working solution: Dilute 20ml of 50X Tris EDTA buffer to 980 ml of DI water.

SPECIMEN PREPARATION

Routinely processed, FFPE tissues are suitable for use with this retrieval buffer. The recommended tissue fixative is 10% neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification. Thickness of the sections should be 2-5µm.

PRECAUTIONS

1. The product is azide free, for further details refer MSDS
2. Do not use reagents after expiration date
3. Use protective clothing and gloves, while handling reagents
4. Avoid microbial contamination of reagents as it may lead to incorrect results

RETRIEVAL PROCEDURE

1. Deparaffnize and rehydrate the tissue sections, block endogenous peroxidase if required and wash with DI water.
2. Dilute the retrieval solution to 1:50 and place the slides into 1X retrieval solution.
3. Retrieve tissue sections under pressure using PathnSitu's Multi Epitope Retrieval System (MERS) for a desired period of time, refer MERS operating manual for detailed protocol.
4. Gently remove the retrieved tissue sections and allow it to cool to room temperature, wash with buffer or DI water and proceed with IHC staining.

QUALITY CONTROL

A positive control slide, which has been treated with the same antigen retrieval conditions as the tissues of interest, should be included in every experiment.

PERFORMANCE CHARACTERISTICS

PathnSitu products will undergo a through quality control check before its release to the market. The retrieval buffer showed consistent results with different antibodies when used within a single run, between runs, between lots and wherever applicable. The products have been determined to be stable for the periods specified on the labels either by standard real time or accelerated testing methods.

TROUBLESHOOTING

1. Evaporation can cause the slide to dry out during AR; increase the volume of antigen retrieval solution in order to cover the slides more completely
2. Run a control experiment to determine the optimal amount of time for antigen retrieval. Use slides of the same tissue section and run the retrieval for 5, 10, 15, 20, 25 and 30 minutes before staining to evaluate optimal antigen retrieval time for the antibody being used.
3. Allow the slides to cool completely before handling. This allows the antigenic sites to re-form after being exposed to high temperature.
4. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com.

LIMITATIONS AND WARRANTY

Authorized and skilled personnel only may use the product. 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.There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

BIBLIOGRAPHY

1. Shi, S.R., et al. Antigen Retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 39:741, 1991.
2. Gown, A.M., et al. Microwave-based antigenic unmasking: a revolutionary new technique for routine immunohistochemistry. Appl. Immunohistochem 1:256-266, 1993.
3. Shi, S.R., et al. Antigen Retrieval technique: a novel approach to immunohistochemistry on routinely processed tissue sections. Cell Vision 2:6-22, 1995.
4. Shi, S.R., et al. Antigen Retrieval immunohistochemistry under the influence of pH using monoclonal antibodies. J Histochem Cytochem 43:193-201, 1995.

EXPLANATION OF SYMBOLS

LOT **- Lot number / Batch number**

- Expiry

IVD ***In vitro* diagnostic use**

Storage limitation

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