Tris-EDTA Buffer (50X Concentrated)

PRODUCT INFORMATION:

REF

PS009 100ml

PS009 500ml

INTENDED USE

For *laboratory* use only

Tris-EDTA buffer (50X Concentrated) is intended for use as an epitope retrieval buffer, which unmasks antigenic sites from formaldehyde fixation and allows for subsequent immunostaining. Use of this high pH retrieval buffer will enhance IHC staining.

SUMMARY AND EXPLANATION

PathnSitu offers a 50X concentrated, high-pH (9.0) Tris-EDTA retrieval solution. It is a colourless, odourless, azide-free, clear solution. Formalin or other aldehyde fixation forms protein cross-links that mask antigenic sites in tissue specimens, thereby resulting in weak or false-negative staining for the immunohistochemical detection of specific proteins. The Tris-EDTA-based solution is designed to break protein crosslinks, thereby unmasking antigens and epitopes in formalin-fixed and paraffin-embedded tissue sections, which enhances the 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 several 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 results.

REAGENT PROVIDED

The reagent is supplied in 100ml and 500ml formats of Tris-EDTA Buffer (50X concentrated).

STORAGE AND HANDLING

Storage Recommendations: Store at 2-8°C. When stored under the appropriate conditions, the retrieval buffer remains stable until expiry. **Do not use the buffer after the expiration date provided on the container.**

To ensure proper reagent performance and stability, replace the dispenser cap after each use. Immediately place the vials in the recommended storage conditions, keeping them away from sunlight and heat, and store them in an upright position.

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 Tris-EDTA (50X Concentrated) Buffer to 980ml 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 due to prolonged fixation or special processes, such as decalcification. Thickness of the sections should be 2-5µm.

PRECAUTIONS

1. This product should be used by qualified and trained professional users only.
2. The product is azide-free. For further details, refer to the MSDS
3. Dispose of waste observing all local, state, provincial or national regulations.
4. Do not use reagents after the expiration date.
5. Use protective clothing or laboratory aprons while handling reagents.
6. Avoid contamination of reagents, as it may lead to incorrect results.

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RETRIEVAL PROCEDURE

1. Deparaffinize and rehydrate the tissue sections.
2. Dilute the retrieval solution to 1:50 and place the slides into the 1X retrieval solution.
3. Retrieve tissue sections under pressure using PathnSitu's Multi Epitope Retrieval System (MERS) for a desired period. Refer to the 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 thorough quality control check before their release to the market. The retrieval buffer showed consistent results with different antibodies when used within a single run, between runs, and between lots, 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. Follow the specific protocol recommendations according to data sheet provided.
2. Gently mix all the reagents prior to use.
3. Evaporation can cause the slide to dry out during the antigen retrieval process; increase the volume of the antigen retrieval solution to cover the entire slide.
4. 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.
5. Allow the slides to cool completely before handling. This allows the antigenic sites to re-form after being exposed to high temperature.
6. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com.

LIMITATIONS AND WARRANTY

1. This product is intended for use only by authorised, trained, and qualified personnel.
2. A qualified and trained pathologist/personnel must interpret the results of the test.
3. Interpretation of test results must be made in conjunction with relevant background information and additional laboratory findings.
4. Always use the recommended volume and concentration of reagents to ensure complete coverage of the tissue section and to minimise the risk of false-positive or false-negative results.
5. Use appropriate buffers, instruments, consumables, and incubation conditions as recommended to achieve optimal staining performance.
6. It is strongly recommended to include known positive and negative controls when performing the test to ensure the validity of results.
7. The product has been validated on formalin-fixed, paraffin-embedded (FFPE) tissues. The end user must establish performance on other tissue types.
8. Unexpected results may occur in untested tissues due to inherent variability in tissue components.
9. False-positive reactions may occur due to insufficient washing, inappropriate protocol conditions, or other contributing factors.
10. Maintain the product under the recommended storage conditions to preserve reagent stability and performance.
11. Do not use reagents that appear cloudy, discoloured, or show signs of contamination. Discard any components showing signs of deterioration.
12. PathnSitu makes no warranties beyond those expressly stated in the product description.
13. PathnSitu shall not be liable for property damage, personal injury, time or effort, or economic loss arising from the use of this product.
14. Please refer to the complete datasheet for all instructions, precautions, and additional product limitations.
15. For detailed information and specifications on individual components, please refer to Product Material Safety Data Sheet (MSDS)

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Laboratory Use Only

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 number / Batch number

Expiry

Storage limitation

Manufacturer address

Date of manufacture

Catalogue number

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