Monosan® HIER T-EDTA Buffer pH 9.0 (10 X)

REF / Cat. No.:	*MON-APP163*	*100 ml (for 1000 ml)*
	MON-APP186	*500 ml (for 5000 ml)*

Instructions for use

Intended use

Monosan® HIER T-EDTA Buffer pH 9.0 is a solution developed for heat induced epitope retrieval (HIER) in formalin-fixed paraffin-embedded tissue sections on slides. This procedure is primarily used in immunohistochemistry.
Monosan® HIER T-EDTA Buffer pH 9.0 is for research use only, not for drug, diagnostic or other use.

Summary and explanations

Immunohistochemical staining procedures consists of sequential incubation steps with blocking solutions, antibodies and secondary reagents, enzymes and chromogenic substrates carried out on tissue sections. These tissue sections are mostly prepared out of formalin-fixed paraffin-embedded tissue blocks. Cellular structures are very effectively stabilised by formalin fixation which results in optimal morphological preservation of the sample. On the other hand the formalin fixation leads to strong cross-links between proteins. This means that epitopes of antigens are being masked and often are no longer accessible for primary antibodies. In order to enable primary antibodies to bind to antigens the epitopes have to be recovered. Heat induced epitope retrieval (HIER) in buffer solutions of different compositions and pH-values restore structures of the epitopes making them more accessible to specific antibodies. Enzymatic digestion with proteolytic enzymes is another way of recovering epitopes. The primary antibody used determines the appropriate method.

Principle of the method

Monosan® HIER T-EDTA Buffer pH 9.0 is a 10fold concentrated EDTA solution in Tris buffer with additives of detergent and stabilising substances. For preparation of the working strength solution the buffer concentrate is diluted 1:10 with deionised or distilled water. The resulting solution has a pH of 9.0 (8.8 to 9.2). Monosan® HIER T-EDTA Buffer pH 9.0 is a very efficient epitope retrieval solution in immunohistochemical staining procedures to be used with primary antibodies of many different specificities. It leads to considerably stronger signals compared with usually used citrate buffer.

Reagents provided

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100 ml Monosan® HIER T-EDTA Buffer pH 9.0

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(10fold concentrated, adequate for 1 litre ready-to-use T-EDTA Buffer)

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500 ml Monosan® HIER T-EDTA Buffer pH 9.0
(10fold concentrated, adequate for 5 litres ready-to-use T-EDTA Buffer)

Storage and handling

The solution should be stored at 2-8°C. Do not freeze it. Under these conditions the solution is stable up to the expiry date indicated on the label. Do not use product after the expiry date. If stored at room temperature the solution is stable for at least 10 month from the date of delivery. The prepared working strength solution is stable for 1 month, if stored at 2-8°C. A positive and a negative control have to be carried out in parallel to the test material. If you observe unusual staining or other deviations from the expected results which could possibly be caused by this reagent, please contact Monosans' technical support or your local distributor.

Precautions

Use by qualified personnel only. Wear protective clothing to avoid contact of reagent and specimen with eye, skin or mucous membranes. In case of reagent or specimen coming into contact with a sensitive area, wash area with large amounts of water. Microbial contamination of the reagent must be avoided, since otherwise non-specific staining may occur. ProClin 300 used for stabilisation is not considered hazardous material in the concentration used. Material safety data sheet (MSDS) is available upon request.

Reagent preparation

Preparation of the T-EDTA buffer working strength solution:

- Dilute Monosan® HIER T-EDTA Buffer concentrate 1:10 with deionised or distilled water and mix thoroughly.
- The pH-value should be at 9.0 (8.8 to 9.2). If necessary adjust pH-value with diluted NaOH or HCl solution.

Procedure

Monosan® HIER T-EDTA Buffer is suitable for various HIER-methods such as steamer, pressure cooker, autoclave, water bath, and microwave oven. Tissue sections used in heat induced epitope retrieval should always be placed on adhesive slides. Epitope retrieval is carried out after dewaxing and rehydration of the sections.

Exemplary protocol using steamer:

1. Prepare the working strength solution by diluting the buffer concentrate as described above and transfer to a Coplin jar. Please make sure that there is enough volume to cover the tissue sections on the slides completely.
2. Fill steamer with water according to instruction manual, close lid and start.
3. After 10 minutes place Coplin jar with T-EDTA buffer in the steamer, close the lid and heat the solution for 20 minutes.

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4. Place slides with tissue sections into the preheated solution and close the lid. Tissue sections have to be completely covered with T-EDTA buffer solution.
5. Incubate slides 20 – 40 minutes. The optimal incubation time needs to be elaborated by the operator.
6. After the incubation take the Coplin jar with slides out of steamer and let cool down at room temperature for about 20 minutes.
7. Remove T-EDTA buffer, rinse slides with wash buffer and proceed with immunohistological staining.

Quality control

We recommend carrying out a positive and a negative control with every staining run. The positive control permits the validation of appropriate processing of the sample. If the negative control has a positive result, this points to unspecific binding. Please refer to the instructions of the detection system for guidance on general quality control procedures.

Troubleshooting

If you observe unusual staining or other deviations from the expected results please read these instructions carefully, contact Monosans' technical support or your local distributor. Also refer to the instructions of the detection systems for guidance on general troubleshooting.

Expected results

During the reaction of the substrate with horse radish peroxidase or alkaline phosphatase in the presence of a chromogen, a coloured precipitate is formed at the location of the bound primary antibody. This reaction only takes place if the target antigen is existent in the tissue. The chromogen used determines the colour of the precipitate. The analysis is carried out using a light microscope.

Limitations of the procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. It requires a highly trained histotechnologist. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (Nadji and Morales, 1983). Inadequate counterstaining and mounting can influence the interpretation of the results.
Monosan® guarantees that the product will meet all requirements described from its shipping date until its expiry date, as long as the product is correctly stored and utilized. No additional guarantees can be given. Under no circumstances shall Monosan® be liable for any damages arising out of the use of the reagent provided.

Performance characteristics

Monosan® has conducted studies to evaluate the performance of the reagent. The product has been found to be suitable for the intended use.

Bibliography

Miller RT et al. Appl Immunohistochem Mol Morphol 8:228-235, 2000
Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983

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Explanation of the symbols on the product label

REF	Bestellnummer Catalog Number Reference du catalogue	LOT	Chargenbezeichnung Batch Code Code du lot	Xi	Reizend Irritant Irritant
Xn	Gesundheitsschädlich Harmful Nocif		Giftig Toxic Toxique		Hersteller / Manufacturer / Fabricant Monosan® Frontstraat 2c 5405 PB Uden The Netherlands Tel: (+31) 413 251115 Fax: (+31) 413 266605 info@monosan.com www.monosan.com
	Verwendbar bis Use By Utiliser jusque				
	Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation		Lagerungstemperatur Temperature Limitation Limites de température		

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