

Pepsin Solution

REF / Cat. No.: ZUC038-060 60 ml

Instructions for use

Intended use

Pepsin Solution is a ready-to-use solution developed for enzymatic epitope retrieval on formalin-fixed tissue sections on slides. This procedure (sometimes called PIER, *Protease Induced Epitope Retrieval*) is primarily used in immunohistochemical staining procedures.

Pepsin solution is intended for research use only.

Summary and explanations

Immunohistochemical staining procedures consist 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.

Enzymatic digestion with proteolytic enzymes (PIER) restores structures of the epitopes making them more accessible to specific antibodies. Heat induced epitope retrieval (HIER) in buffer solutions of different compositions and pH-values is another way of recovering epitopes. The primary antibody used determines the appropriate method.

Principle of the method

Pepsin Solution is a ready-to-use stabilised pepsin solution for enzymatic epitope retrieval.

Reagent provided

REF / Cat. No. ZUC038-060
60 ml Pepsin Solution (ready-to-use)

Storage and handling

The solution should be stored at 2-8°C without further dilution. 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.

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. A material safety data sheet (MSDS) is available upon request.

Reagent preparation

The solution is ready-to-use.

Procedure

Pepsin Solution is suitable for enzymatic epitope retrieval carried out after the dewaxing and rehydration of the tissue sections.

1. Cover deparaffinised and rehydrated tissue sections with ready-to-use Pepsin Solution.
2. Incubate for 10 - 15 minutes at 37°C or 20 – 30 minutes at room temperature. The optimal incubation time needs to be elaborated by the operator. It was shown that in individual cases a stronger signal can be obtained when the incubation time is elongated up to 120 minutes (e. g. for detection of Collagen IV with different antibodies).
3. Rinse carefully (3 x) with wash buffer.
4. Proceed with immunohistological staining as usual.

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 staining. 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 Zytomed Systems' 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. Zytomed Systems 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 Zytomed System be liable for any damages arising out of the use of the reagent provided.

Performance characteristics

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

Bibliography

Varma M, Linden MD, Amin MB Mod Pathol 12:472-478, 1999
Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983



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Explanations of the symbols on the product label

Symbols are used in accordance with ISO 15223-1. Further symbols on the product label might be:



GSH02: Flammable



GSH05: Caustic



GSH07: Attention / Warning



GSH08: Systemic health hazards

RUO

For Research Use Only