**Product datasheet** **MON-APP157**

**Monosan Trypsin Pretreatment Kit, 155 ml (125 ml Buffer 30 ml Enzym)**

Reagents

**Instructions for use**

**Product name**

Monosan Trypsin Pretreatment Kit, 155 ml (125 ml Buffer 30 ml Enzym)

**Intended Use**

Trypsin Pretreatment Kit consists of 2 reagents for the preparation of a trypsin solution used 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. Trypsin Pretreatment Kit is for research use only.

**Applications**

IHC-P, IHC-Fr, IF

**Summary and explanation**

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.

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**Principle of method**

The components of this Trypsin Pretreatment Kit allow for the preparation of a buffered trypsin solution for enzymatic epitope retrieval.

**Reagents provided**

30 ml Trypsin Solution 125 ml Trypsin Buffer

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## Storage and handling

The solutions should be stored at 2-8°C. Please sto re the reagents in a dark place and do not freeze them. Under these conditions the solutions are stable up to the expiry date indicated on the label. Do not use product after the expiry date. 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 our technical support.

## Reagent preparation

Mix 1 part Trypsin Solution with 3 parts Trypsin Buffer.
• The activity of the resulting trypsin solution can be adjusted by variation of the mixing ratio. Mix the two components in the ratio 1:1 when strong epitope retrieval is desired.
• The working solution is stable for at least one week if stored at 2-8°C.

## Procedure

Trypsin Pretreatment Kit is suitable for enzymatic epitope retrieval carried out after the dewaxing and rehydration of the sections.
1. Cover deparaffinised and rehydrated tissue sections with trypsin working solution.
2. Incubate for 10 - 20 minutes at 37°C.
3. Rinse carefully (3 x) with wash buffer.
4. Proceed with immunohistological staining as usual.

## 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.

## Trouble shooting

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

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## 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.

## Performance

Studies have been conducted to evaluate the performance of the kit reagents. The product has been found to be suitable for the intended use

## Limitations of 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. Sanbio 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 Sanbio be liable for any damages arising out of the use of the reagent provided.

## 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.

## References

1. Ashton-Key M, Jessup E, Isaacson PG Histopathol 29(6):525-31, 1996
2 Cattoretti G, Pileri S, Parravicini C et al. J Pathol 171(2):83-98, 1993
3. Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-139, 1983

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