Trypsin pretreatment, Kit

Reference: AP12204

INTENDED USE AND PRESENTATION:

For research use only.
AP12204. 155 mL.

SUMMARY, EXPLANATION AND LIMITATIONS:

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. 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. 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.
Immunohistochemistry (IHC) is a complex technique in which immunological and histological detection methods are combined. In general, the manipulation and processing of tissues before immunostaining, especially different types of tissue fixation and embedding, as well as the nature of the tissues themselves may cause inconsistent results (Nadji and Morales, 1983).

APPLICATIONS:

Trypsin pretreatment Kit is intended for used for enzymatic epitope retrieval on formalin-fixed tissue sections on slides. This procedure (sometimes called PIER) is primarily used in immunohistochemical staining procedures.
The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.

REAGENT PROVIDED:

30 mL. **Trypsin Solution**
125 mL. **Trypsin Buffer**

METHOD AND PROCEDURE:

Principle of the method: The IHC as technique to demonstrate the presence of an antigen in tissues and cells, is a sequential procedure of several steps: the application of antibody specific for the antigen of interest (primary antibody), the detection and visualization of bound antibody by one of a variety of enzyme chromogenic systems and washing steps. The chromogenic enzyme activation results in a visible product at the site where the antigen is located. The results can be evaluated in a light microscope.
Specimen: Formalin-fixed paraffin-embedded tissue section.
Reagent preparation: Preparation of the working solution:
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.

See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for IHC procedures are not provided with this product. This includes adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H_2O, heat pretreatment equipment (pressure cooker, steamer, microwave), pipettes, Coplin jars, glass jars, moist chamber, histological baths, negative control reagents, counter-staining solution, mounting materials, and microscope.
Buffered solutions for antigen retrieval, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Gennova Scientific.

STORAGE AND STABILITY:

Store at 2-8 °C until the expiration date printed on product label. Please store the reagents in a dark place and do not freeze them. Do not use after the expiration date. If fresh solutions are required, these must be prepared immediately prior to use. Unused portion of working solution should be discarded. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user.
Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances is Gennova Scientific obliged to cover damages caused by use of this reagent.

TROUBLESHOOTING:

If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the detection system. If this does not solve the problem, please contact Gennova Scientific's technical support department or your local distributor.

PRECAUTIONS:

Use only by qualified personnel.

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Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific staining results. Material safety data sheet (MSDS) is available upon request.

PERFORMANCE CHARACTERISTICS:

Gennova Scientific has performed studies to evaluate the functioning of the kit for use with standard detection systems, concluding that the product has been found to be suitable for the intended use.

BIBLIOGRAPHY:

Elias JM "Immunohistopathology – A practical Approach to Diagnosis" ASCP Press 2003.
Nadji M, Morales AR. Immunoperoxidase, part 1: the techniques and its pitfall. Lab Med 1983; 14:767-770.

REF Catalog number | LOT Batch code | RUO Research use only

Temperature limitation | Expiration date

Manufacturer | See instruction for use

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