

Pepsin

Reference: AP12200



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INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.

AP12200. 60 mL. Ready to use

SUMMARY, EXPLANATION AND LIMITATIONS:

Pepsin Solution is 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. 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:

Pepsin Solution is developed for enzymatic epitope retrieval on formalin-fixed tissue sections on slides. This procedure 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:

60 mL **Pepsin Solution**, Ready to use.

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: Pepsin solution is ready-to-use and should be at room temperature prior 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.
- 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 H₂O, 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:

The solution should be stored at 2-8°C without further dilution until the expiration date printed on product label. Do not freeze it. Do not use after the expiration date. 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.

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



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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nonspecific staining results. Material safety data sheet (MSDS) is available upon request.

PERFORMANCE CHARACTERISTICS:

Genova 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.

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