

# Proteinase K

References: AP12202 / AP12203



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## INTENDED USE AND PRESENTATION:

For research use only.

**AP12202, 15 mL**

**AP12203, 50 mL**

## SUMMARY, EXPLANATION AND LIMITATIONS:

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 stabilized 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.

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.

## APPLICATIONS:

Proteinase K 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.

Formalin fixation leads to a strong cross-linking between proteins. This means that the antigenic epitopes being masked and sometimes are not well accessible to the primary antibody. For the antibody binds to its antigen, epitopes have to be retrieved.

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:

**Ref.: AP12202**

15 ml **Proteinase K Solution** (Ready-to-use).

**Ref.: AP12203**

50 ml **Proteinase K 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:** Proteinase K Solution is a ready-to-use Proteinase K solution in TBS buffer for enzymatic epitope retrieval.

**Procedure:** Proteinase K Solution is suitable for enzymatic epitope retrieval carried out after the dewaxing and rehydration of the tissue sections.

1. Apply 2-3 drops of ready-to-use Proteinase K Solution to deparaffinised and rehydrated tissue sections.

2. Incubate for 15 - 10 minutes at room temperature. The optimal incubation time needs to be elaborated by the operator.

3. Rinse carefully (3 x), first with distilled water followed by buffer.

4. Proceed with immunohistological staining as usual.

See our web site at [www.gennova-europe.com](http://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 primary antibodies, adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H<sub>2</sub>O, 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.

Antibodies, buffered solutions for antigen retrieval, enzyme treatments, others highly sensitive detection systems, and other auxiliary reagents are available from Genova Scientific.

## STORAGE AND STABILITY:

Store at -20 °C in aliquots without further dilution. 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.

Genova 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 Genova Scientific obliged to cover damages caused by use of this reagent.



Catalog number



Batch code



Research use only



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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## **TROUBLESHOOTING:**

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

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

## **PERFORMANCE CHARACTERISTICS:**

Genova Scientific has conducted studies to evaluate the performance of the kit reagents. 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

Omata M et al. Am J Clin Pathol 73(5): 626-32, 1980

Nadji M, Morales AR. Immunoperoxidase, part 1: the techniques and its pitfall. Lab Med 1983; 14:767-770.

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**REF**

Catalog number

**LOT**

Batch code

**RUO**

Research use only



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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