HIER Citrate Buffer pH 6.0

(10 X Concentrated)

References: AP12100; AP12101

INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.
AP12100, 100 mL. (10 X Concentrated)
AP12101, 500 mL. (10 X Concentrated)

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 the antigens the epitopes have to be recovered. Heat induced epitope retrieval (HIER) in buffer solutions of different compositions and pH-values restore structures of the epitopes making them more accessible to specific antibodies. Enzymatic digestion with proteolytic enzymes 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:

HIER Citrate Buffer pH 6.0 is a solution developed for heat induced epitope retrieval (HIER) in formalin-fixed paraffin embedded tissue sections on slides. This procedure is primarily used in immunohistochemistry.
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.: AP12100
100 ml HIER Citrate Buffer pH 6.0
(10fold concentrated, adequate for 1 litre of ready-to-use citrate buffer)
Ref.: AP12101
500 ml HIER Citrate Buffer pH 6.0
(10fold concentrated, adequate for 5 litres of ready-to-use citrate 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.
HIER Citrate Buffer pH 6.0 is a 10fold concentrated citrate buffer solution with additives of stabilising substances. For preparation of the working strength solution the buffer concentrate is diluted 1:10 with deionised or distilled water. The resulting solution has a pH of 6.0 (5.8 to 6.2). HIER Citrate Buffer pH 6.0 is a very efficient epitope retrieval solution in immunohistochemical staining procedures to be used with primary antibodies of many different specificities.
Specimen: Formalin-fixed paraffin-embedded tissue section.
Reagent preparation: Preparation of the citrate buffer working strength solution:
- Dilute HIER Citrate Buffer concentrate 1:10 with deionised or distilled water and mix thoroughly.
- The pH-value should be at 6.0 (5.8 to 6.2). If necessary adjust pH-value with diluted NaOH or HCl solution.

Procedure: HIER Citrate Buffer is suitable for various HIER-methods such as steamer, pressure cooker, autoclave, water bath, and microwave oven. Tissue sections used in heat induced epitope retrieval should always be placed on adhesive slides.
Epitope retrieval is carried out after dewaxing and rehydration of the sections.
Exemplary protocol using steamer:
1. Prepare the working strength solution by diluting the buffer concentrate as described above and transfer to a Coplin jar. Please make sure that there is enough volume to cover the tissue sections on the slides completely.
2. Fill steamer with water according to instruction manual, close lid and start.
3. After 10 minutes place Coplin jar with citrate buffer in the steamer, close the lid and heat the solution for 20 minutes.
4. Place slides with tissue sections into the preheated solution and close the lid. Tissue sections have to be completely covered with citrate buffer solution.
5. Incubate slides 20 – 40 minutes. The optimal incubation time needs to be elaborated by the operator.
6. After the incubation take the Coplin jar with slides out of steamer and let cool down at room temperature for about 20 minutes.
7. Remove citrate buffer, rinse slides with wash buffer and proceed with immunohistological staining.
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 primary antibodies, adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H_2O, heat pretreatment equipment

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(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 Gennova Scientific.

STORAGE AND STABILITY:
Store at 2-8 °C without further dilution and do not freeze it. Do not use after the expiration date. If stored at room temperature the solution is stable for at least 10 month from the date of delivery. The prepared working strength solution is stable for 1 month, if stored at 2-8°C. 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 you observe unusual staining or other deviations from the expected results please read these instructions carefully, contact Gennova 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. ProClin 300 used for stabilisation is not considered hazardous material in the concentration used. Material safety data sheet (MSDS) is available upon request.

PERFORMANCE CHARACTERISTICS:
Gennova 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:
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Shi S-R, Key ME, Kalra KL J Histochem Cytochem 39:741-748, 1991
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Nadji M, Morales AR. Immunoperoxidase, part 1: the techniques and its pitfall. Lab Med 1983; 14:767-770.

REF Catalog number
LOT Batch code
IVD In Vitro diagnostic medical device
Temperature limitation
Expiration date
Manufacturer
See instruction for use

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