

Permanent AEC Kit

(For permanent mounting)

Reference: AP13000



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INTENDED USE AND PRESENTATION:

For research use only.

AP13000. 2000 Tests.

SUMMARY, EXPLANATION AND LIMITATIONS:

Permanent AEC Kit is intended for immunohistochemical and in situ-hybridisation staining procedures with HRP. AEC (3-Amino-9-ethylcarbazol) leads to the formation of a red-brown precipitate at the location of the target antigen or target nucleic acid. The precipitate is insoluble in aqueous and organic solvents and can be observed by light microscopy.

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). In some tissues endogenous peroxidase activity may cause non-specific staining. The enzyme activity should be blocked by incubation with hydrogen peroxide solution (H₂O₂ solution, REF AP12002/AP12003). The step is carried out before incubation with primary antibody but after dewaxing and rehydration. Background staining due to endogenous biotin can be blocked through an avidin-biotin blocking step prior to the primary antibody incubation step. Inadequate counterstaining and mounting can influence the interpretation of the results.

A longer exposure to absolute ethanol can result in decreasing staining intensity. Use of recycled alcohol to dehydrate tissue slides after staining is not recommended.

APPLICATIONS:

Permanent AEC Kit is intended for immunohistochemical and in situ-hybridisation staining procedures with horse radish peroxidase (HRP).

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:

| | |
|--------|--|
| 5.5 ml | Reagent 1 |
| 3 ml | Reagent 2 |
| 3 ml | Reagent 3 (Chromogen) |
| 4.5 ml | Reagent 4 (H ₂ O ₂) |
| 1 | Dilution Vial |

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:

1) Pipette 5 ml distilled or deionised water into the provided dilution vial.

2) Add 3 drops buffer concentrate (Reagent 1). Mix thoroughly.

3) Add 2 drops Reagent 2. Mix thoroughly.

4) Add 2 drops AEC chromogen (Reagent 3). Mix thoroughly.

5) Add 2 drops H₂O₂ substrate (Reagent 4). Mix thoroughly.

This working solution is stable for at least 16 hours if stored at 2-8°C in a dark place.

Procedure: 1) Apply the Permanent AEC working solution onto the slide. Incubate for 5-15 minutes. (Incubation time can be extended, if desired.)

2) Rinse with distilled or deionised H₂O.

3) Counterstain with haematoxylin for about 30 seconds up to 5 minutes (depending on the desired staining intensity).

4) Rinse with distilled or deionised H₂O.

5) Blueing in tap water for at least 5 minutes.

6) Dehydrate through a graded series of ethanol and clear in xylene. Mount with a permanent mounting medium.

Note: It is also possible to mount Permanent AEC with aqueous mounting media. However, slides mounted in a permanent mounting medium after dehydration in xylene show a much more brilliant 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 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 Genova Scientific.

STORAGE AND STABILITY:

Store at 2-8 °C until the expiration date printed on product label. Do not use after the expiration date. If fresh solutions are required, these must be prepared immediately prior to use, and will be stable for at least 16 hours if stored at 2-8°C in a dark place. Unused portion of working solution should be discarded after this time. 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



Catalog number



Batch code



Research use only



Temperature limitation



Expiration date



Test number



Manufacturer



See instruction for use



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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 Genova 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 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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