

AEC Substrate Kit

(For aqueous mounting)

Reference: AP13003



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INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.

AP13003. 55 mL / 500 Tests.

SUMMARY, EXPLANATION AND LIMITATIONS:

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 mounting media and can be observed by light microscopy. AEC Substrate kit is intended for immunohistochemical and *in situ*-hybridisation staining procedures with horse radish peroxidase (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 mounting media 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. The coloured precipitate formed by AEC is soluble in organic solvents. The tissue sections therefore have to be counterstained with aqueous solutions (e. g. Gill's or Mayer's haematoxylin) and mounted with aqueous mounting media (REF AP12011). The colour intensity of the reaction product can decrease with time, especially when exposed to light. The staining reaction itself can be influenced in the same way when carried out in strong light.

APPLICATIONS:

AEC Substrate kit is intended for immunohistochemical and *in situ*-hybridisation staining procedures with 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:

3 ml **AEC Chromogen** (liquid AEC concentrate)

11 x 5 ml **AEC Substrate 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.

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 mounting media and can be observed by light microscopy. AEC Substrate kit is especially useful when a high sensitivity is desired.

Specimen: Formalin-fixed paraffin-embedded tissue section.

Reagent preparation: Add 2 drops of AEC Chromogen (AEC concentrate) to one bottle of AEC Substrate Buffer and mix thoroughly.

Procedure:

- 1) Rinse the slide with wash buffer after the previous incubation step.
- 2) Apply the AEC working solution onto the slide. Incubate for 5-20 minutes.
- 3) Rinse with distilled H₂O.
- 4) Counterstain with haematoxylin for about 30 seconds up to 5 minutes (depending on the desired staining intensity).
- 5) Rinse with distilled H₂O.
- 6) Blueing in tap water for at least 5 minutes.
- 7) Mount with an aqueous mounting medium.

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 solutions should be stored at 2-8 °C without further dilution. Please store the reagents in a dark place and do not freeze them. Under these conditions the solutions are stable up to the expiry date indicated on the label. Do not use product after the expiry date. The working solution should be prepared freshly at the day of use. Once the two reagents are combined, the resulting solution is stable for up to three hours. Excess working solution needs to be disposed as hazardous substance. 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



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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used as recommended. No other guarantees are provided. Under no circumstances is Genova 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 Genova Scientific's technical support department or your local distributor.

PRECAUTIONS:

Use only by qualified personnel.

Wear protective clothing to avoid contact of reagent or specimen with eye, skin or mucous membrane. In case of reagent or specimen coming into contact with a sensitive area, wash the area with large amounts of water. Oxidising substances, e. g. metals, dust, bacteria or glass devices can influence the stability of AEC. Such contaminations have to be avoided. Material safety data sheet (MSDS) for pure AEC 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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