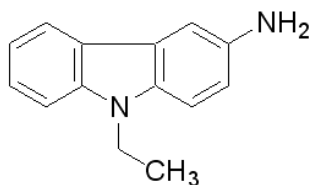


Product Information

AEC Staining Kit

AEC101

Product Description



3-Amino-9-ethylcarbazole (AEC) is a chromogen that is suitable for use with horseradish peroxidase (HRP) conjugates in immunoblotting and immunohistochemistry staining procedures. The reaction of AEC with HRP produces an insoluble end product that is red in color and can be observed visually, in 0.05 M acetate buffer at pH 5.^{1,2}

Several theses³⁻⁶ and dissertations⁷⁻¹² have cited use of product AEC101 in their protocols.

Reagents

- Vial 1, Acetate Buffer: Acetate buffer, 2.5 M, pH 5.0
- Vial 2, AEC Chromogen: 3-amino-9-ethylcarbazole (AEC) in *N,N*-dimethylformamide (DMF)
- Vial 3, Hydrogen Peroxide: 3% H₂O₂ in deionized water

Storage/Stability

Store this product at 2–8 °C.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Usage

Immunohistochemistry

After application of a peroxidase reagent, wash the slides. Carefully wipe around the tissue section. The following steps are carried out at room temperature.

1. Prepare AEC Substrate Reagent in a test tube or mixing vial shortly before incubation. In order, add:
 - 4 mL deionized water
 - 2 drops Acetate Buffer (Vial 1)
 - 1 drop AEC Chromogen (Vial 2)
 - 1 drop 3% hydrogen peroxide (Vial 3)
2. Apply 2 drops of AEC Substrate Reagent to each slide.
 - 2.1. Incubate up to 10 minutes.
 - 2.2. Check the slide microscopically for adequate chromogen development.
3. When sufficient staining has been achieved, rinse the slides in deionized water for 5 minutes. Carefully wipe the slide around the section.

Note: Do not use alcohol-containing solutions for counterstaining, since the AEC stain formed by this method is soluble in organic solvents.

Expected Observations: Cytoplasm of positive cells will be rose-red to brownish-red.

Immunoblot or Dot Blot

After incubation with a peroxidase reagent, wash and rinse the strips. The following steps are carried out at room temperature.

1. Prepare AEC Substrate Reagent in a test tube or petri dish shortly before incubation. In order, add:
 - 4 mL deionized water
 - 2 drops Acetate Buffer (Vial 1)
 - 1 drop AEC Chromogen (Vial 2)
 - 1 drop 3% hydrogen peroxide (vial 3)
2. Add strips to AEC Substrate Reagent. Incubate for 5-10 minutes until a clear red insoluble signal is obtained for the positive control.
3. Wash the strips in several changes of deionized water.

Expected Observations:

- Rose-red insoluble precipitates in the form of bands or dots.
- The nitrocellulose strip will normally have a slight reddish background.

References

1. Graham, R.C., Jr. *et al.*, *J. Histochem. Cytochem.*, **13(2)**, 150-152 (1965).
2. Kaplow, L.S., *Am. J. Clin. Path.*, **63(3)**, 451 (1975).
3. Torres, Donna Schulz, "Effects of Modulus of Elasticity of Collagen Sponges on Their Cell-Mediated Contraction *In Vitro*". Massachusetts Institute of Technology, M.S. thesis, p. 34 (1998).
4. Harley, Brendan A., "Peripheral Nerve Regeneration through Collagen Devices with Different *In Vivo* Degradation Characteristics". Massachusetts Institute of Technology, M.S. thesis, p. 149 (2002).
5. Tang, Tianyi, "Regulation of adipose tissue metabolism by NFκB P65 in transgenic mice". Louisiana State University, M.S. thesis, p. 17 (2009).
6. Orvis, Emily, "Immunobiology of Adenovirus-Vector Vaccines for MRSA ". Loyola University Chicago, M.S. thesis, p. 38 (2018).
7. Kousar, Soumira, "Recherche et caractérisation de glycosyltransférases impliquées dans la biosynthèse des polysaccharides de la paroi chez *Arabidopsis thaliana*" ("Research and characterization of glycosyltransferases involved in the biosynthesis of cell wall polysaccharides in *Arabidopsis thaliana*"). Université de Grenoble, Ph.D. dissertation, p. 160 (2011).
8. Stark, Felicity Christana, "The role of CD8⁺ T cell phenotype and cytotoxicity on cancer immunotherapy". University of Ottawa, Ph.D. dissertation, p. 51 (2011).
9. Wisastra, Rosaline, "Modulation of lipoxygenase activity and chemistry-based detection of protein nitration in inflammation". University of Groningen, Ph.D. dissertation, p. 119 (2013).

10. Preuße, Corinna, "Common and distinct immunological aspects in acquired inflammatory myopathies and inherited muscular dystrophy". Humboldt-Universität zu Berlin, Dr. rer. nat. dissertation, p. 18 (2014).
11. Shikatani, Eric Akihiko, "Distinguishing the Role of the *c-myc* Proto-Oncogene in Mouse Models of Cardiovascular Disease". University of Toronto, Ph.D. dissertation, p. 174 (2016).
12. McCabe, William Albert, "The role of PNPLA3 in the development and progression of chronic liver disease". University College London, Ph.D. dissertation, p. 146 (2020).

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