AEC Peroxidase Substrate Kit for IHC (Red Color, 20X)

Introduction

3-Amino-9-ethylcarbazole (AEC) is a chromogenic substrate for peroxidase. Under the catalysis of peroxidase, hydrogen peroxide oxidizes AEC to form a stable red precipitate. This red product is insoluble in water but soluble in organic solvents. This AEC substrate chromogenic kit contains enhanced chromogenic reagents that significantly improve sensitivity. The product is easy to use, produces clear coloration, and offers good reproducibility. It is suitable for enzyme-based color development in HRP-based IHC and Western blot experiments.

Components and Storage

Components	1 mL	10 mL	Storage
Solution A Concentrate (20X)	1 mL	10 mL	4°C away from light
Solution B Concentrate (20X)	1 mL	10 mL	4°C away from light
Solution C Concentrate (20X)	1 mL	10 mL	4°C away from light
Shipping: Blue ice	**Shelf life: 12 months**		

Protocol

1. Preparation of working solution

   Take 850 µL of sterile double-distilled water, then add 50 µL of Solution A, 50 µL of Solution B, and 50 µL of Solution C in sequence. Mix thoroughly to obtain the AEC working solution. If a larger volume is needed, increase all components proportionally. This solution must be freshly prepared before use, protected from light after preparation, and used within 30 minutes; discard any remaining solution after it expires.

2. Color development reaction:

   a. Color development on western blot membranes: Add the prepared working solution onto the membrane (or immerse the membrane in the AEC working solution). The typical development time is 10–30 min. After color development, immerse the membrane in distilled water to stop the reaction.

   b. Staining of tissue sections: Add an appropriate amount of AEC working solution to the tissue section, ensuring the sample is fully covered. Incubate at room temperature for 10–30 min, protected from light, until the desired staining intensity is achieved. The color development time can be monitored and controlled under a microscope. After color development, wash with distilled water to terminate the

reaction.

c. For tissue sections or cell samples, other counterstains may be applied after terminating the color development reaction. For membranes, after terminating the reaction, they can be air-dried at room temperature and stored protected from light.

Note

1. Since the colored product generated by AEC is lipophilic, it is not suitable for dehydration through a graded alcohol series, clearing with xylene, or mounting with neutral mounting medium after IHC color development. An aqueous mounting medium must be used.
2. Optimize experimental conditions and adjust color development time as needed to achieve the best staining results.
3. This product is for research use only.

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