

ImmPACT[™] AEC Peroxidase Substrate Cat. No. SK-4205

Store at 2-8 °C

Burlingame, CA USA

STORAGE:

• Store reagents in original bottles at 2-8 °C.

• Avoid storing reagents or working solution in strong direct light.

PREPARATION OF SUBSTRATE WORKING SOLUTION:

• To 5 ml of **ImmPACT[™] AEC** Diluent:

Add 2 drops (approximately $64 \ \mu l^*$) of **ImmPACT[™] AEC** Reagent 1 Add 3 drops (approximately $90 \ \mu l^*$) of **ImmPACT[™] AEC** Reagent 2 Add 2 drops (approximately $80 \ \mu l^*$) of **ImmPACT[™] AEC** Reagent 3

· Mix well before use

* Drop volumes differ due to solvent compositions.

INSTRUCTIONS FOR USE:

For Tissues and Cells

After incubation with a peroxidase (HRP) detection system, rinse well. Incubate with the substrate working solution at room temperature for 10-30 minutes. Optimal development times should be determined by the investigator.

Wash for 5 minutes in water.

Counterstain, if desired, with Vector[®] Hematoxylin (Cat. No. H-3401) or Vector[®] Hematoxylin QS (Cat. No. H-3404). (See counterstain compatibility chart on reverse side.)

Do not dehydrate. Coverslip with an aqueous mounting medium such as VectaMount[™]AQ (Cat. No. H-5501).

For Blots

Prepare appropriate volume of working solution. Development time is generally 10-20 minutes at room temperature. When signal development is satisfactory, rinse the membrane in water and air dry.

ImmPACT[™] AEC can cause wrinkling of some nitrocellulose membranes.

NOTES:

Unused Working Solution is stable up to 14 days if stored at 2-8 °C. Working solution may change color during storage but this will have no effect on the quality or intensity of the staining. If a precipitate forms, filter through a 0.45 μ m filter.

It is not necessary or recommended to add detergent to **ImmPACT[™] AEC** working solution to reduce surface tension (e.g. for use in an automated stainer).

IMPORTANT: Little is known about the toxicity and carcinogenicity of the substrate kit components. Appropriate care should be exercised when using this reagent including gloves, eye protection, lab coats, and good laboratory procedures. Dispose in accordance with local regulations.

For Laboratory Use

ImmPACT[™] AEC Substrate

ImmPACT[™] AEC Substrate produces a red reaction product in the presence of peroxidase (HRP) enzyme. ImmPACT[™] AEC Substrate can be used on tissue sections or cells, or on membranes such as PVDF or nylon.

The ImmPACT[™] AEC Substrate contains all of the reagents necessary to prepare a working solution of ImmPACT[™] AEC (3-amino-9-ethylcarbazole). These reagents are supplied in convenient dropper bottles with an optimized diluent.

REAGENTS:

120 ml ImmPACT[™] AEC Diluent 3 ml ImmPACT[™] AEC Reagent 1 3 ml ImmPACT[™] AEC Reagent 2 3 ml ImmPACT[™] AEC Reagent 3