



ImmPACT™ Vector® Red

Alkaline Phosphatase

Cat. No. SK-5105

Store at 2-8 °C

Burlingame, CA USA

ImmPACT™ Vector® Red Substrate

The ImmPACT™ Vector® Red Substrate produces a red reaction product in the presence of alkaline phosphatase (AP) enzyme. This reaction product is also highly fluorescent and non-fading and can be viewed with Texas Red® or rhodamine filter systems. The ImmPACT™ Vector® Red Substrate can be used on tissue sections or cells, or on membranes, such as nitrocellulose, PVDF, or nylon.

REAGENTS:

- 120 ml ImmPACT™ Vector® Red Diluent
- 2.5 ml ImmPACT™ Vector® Red Reagent 1
- 2.5 ml ImmPACT™ Vector® Red Reagent 2 (Tween® 20)

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 ImmPACT™ Vector® Red Diluent:
 - Add 2 drops (80 µl*) of ImmPACT™ Vector® Red Reagent 1
 - Add 2 drops (60 µl*) of ImmPACT™ Vector® Red Reagent 2†
- Mix well before use

* Drop volumes differ due to solvent compositions.

† For blots do not add Reagent 2.

INSTRUCTIONS FOR USE:

For Tissues or Cells

After incubation with an AP detection system, rinse well. Incubate with the substrate working solution for 20-30 minutes. Optimal development times should be determined by the investigator. Improved staining may be obtained by developing the substrate in the dark.

Wash slides in buffer for 5 minutes. Rinse in water and counterstain, if desired (see chart on reverse).

For permanent mounting: Dehydrate, clear and mount in a permanent mounting medium, such as **VectaMount™** (H-5000).

For aqueous mounting: Use an aqueous based mounting medium such as **VectaMount™ AQ** (H-5501) or a **VECTASHIELD®** Mounting Medium (H-1000, H-1200, H-1400 or H-1500). To increase the intensity of ImmPACT™ Vector® Red fluorescence, rinse slides 2-5 minutes in 100% ethanol before mounting.

For Blots

Incubate blot for 20-30 minutes. When development is satisfactory, rinse blot in water and air dry.

NOTES:

1. Use the working solution of ImmPACT™ Vector® Red within 15 minutes of preparation.
2. Do not heat ImmPACT™ Vector® Red substrate kit components or working solution.
3. Occasionally a precipitate may form in Reagent 1 upon prolonged storage. This will have no effect on the quality or intensity of the staining. Do not filter the reagents or working solution.
4. Endogenous alkaline phosphatase activity (other than the intestinal isoenzyme) can be inhibited by the addition of **Levamisole** (SP-5000) to Diluent prior to the preparation of the working solution. Alternatively, endogenous alkaline phosphatase, including the intestinal isoenzymes, can be inactivated before immunostaining with the use of **BLOXALL™** Blocking Solution (SP-6000) or with other tissue pretreatments (Bulman AS and Heyderman E; J. Clin. Pathol. 34, 1349-1351, 1981).

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.

Tween® 20 is a registered trademark of Atlas Chemical Industries

Texas Red® is a registered trademark of Molecular Probes Inc.

For Laboratory Use

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