Alkaline phosphatase staining (www.vectorlabs.com)

Vector®Red alkaline phosphatase substrate kit I - Cat. No. SK-5100

The Vector®Red substrate kit contains all of the reagents necessary (except buffer) to prepare a working solution for either immunocytochemical of membrane-based applications. Vector®Red produces a red reaction product that can be seen using either brightfield or fluorescent microscopy. Vector®Red can be permanently mounted in non-aqueous media or mounted aqueously in VECTASHIELD®Mounting Medium.

Dispensing reagents:
For convenience, the reagents are supplied in dropper bottles. When dispensing drops, hold the bottle in an inverted vertical position and squeeze gently. To prevent evaporation, secure the opaque caps on the bottles when they are not in use. DO NOT PIPET REAGENTS DIRECTLY FROM BOTTLES. Drop volumes of each component may be different due to solvent characteristics. Proper concentrations of substrate components in the working solution are assured only by using the drop dispensers.

Instructions for use:
Prepare the Vector®Red substrate working solution immediately before use in the mixing bottle or test tube.
1. To 5 ml of 100 mM Tris-HCl, pH 8.2 - 8.5 buffer*, add 2 drops of Reagent 1. Mix well.
2. Add 2 drops of Reagent 2 and mix well.
3. Add 2 drops of Reagent 3 and mix well.
* It is important to make the working solution in 100 mM - 200 mM Tris-HCl, pH 8.2 - pH 8.5

Incubate tissue sections or membranes with substrate solution at room temperature until suitable staining develops. Development times should be determined by the investigator but generally 20-30 minutes provides good staining intensity. Improved staining may be obtained by developing the substrate in the dark.

Wash sections in assay buffer for 5 minutes. Rinse in water.
For permanent mounting: Rinse in tap water, counterstain if desired (see chart on reverse), dehydrate, clear, and mount in a permanent mounting medium, such as VectaMount TM, Catalog No. H-5000.

For aqueous mounting in VECTASHIELD®Mounting Medium: Tap excess buffer from sections and mount. Before mounting, slides may be rinsed for 2-5 minutes in 100% ethanol to increase the intensity of Vector®Red fluorescence.
The Vector®Red reaction product is a highly fluorescent, non-fading, bright red precipitate when viewed with Texas Red® or rhodamine excitation and emission filter systems. Vector®Red fluorescence may also be visible with fluorescein or AMCA filter systems using broad band emission filters.

**Notes:**

1. Use the working solution of Vector®Red within 15 minutes of preparation or decreased sensitivity may result. Increasing the incubation time in substrate solution beyond 45 minutes will not increase sensitivity, unless freshly made substrate solution is reapplied to sections.

2. **Important.** For tissue, using 0.1% Tween®20 in the Vector®Red working solution may increase the sensitivity and crispness of staining especially when non-enzymatic antigen retrieval methods are used in the immunostaining protocol. Tween®20 should not be added to the Vector®Red substrate solution for membrane applications.

3. Do not heat Vector®Red substrate kit components or working solution. Heating decreases staining sensitivity.

4. The reagents should be stored at 4 °C and protected from light whenever possible. Occasionally a precipitate may form in some reagents upon prolonged storage. This will have no effect on the quality or intensity of the staining. Do not filter the reagents or working solution.

5. Do not put sodium azide in the buffer used for the Vector®Red working solution; it will prevent staining.

6. When using neural tissue, Vector®Red is not recommended for visualizing processes, fibers, or terminals (inadequate staining may occur).

7. Endogenous alkaline phosphatase activity (other than the intestinal isoenzyme) can be inhibited by the addition of levamisole (Cat. No. SP-5000) to the buffer prior to the preparation of the working solution.

Intestinal alkaline phosphatase activity can be inhibited, before immunostaining, with several 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 components. Care should be taken in the handling and disposing of all the reagents. **Tween®20 is a registered trademark of Atlas Chemical Industries**
Undifferentiated hESCs (above) stain for alkaline phosphatase (AP-blue), differentiating hESCs begin to lose AP staining.