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## Vector® *NovaRED*™ SUBSTRATE KIT FOR PEROXIDASE

Catalog Number SK-4800

The Vector® *NovaRED*™ Substrate Kit contains all of the reagents necessary to prepare a working solution for staining tissue sections or nitrocellulose, nylon, or other membranes. Vector® *NovaRED*™ produces a red reaction product.

### **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 are assured in preparing the working solutions by using the drop dispensers only.

### **INSTRUCTIONS FOR:**

#### **Use in Immunohistochemical Staining**

Immediately before use on tissue sections, prepare the substrate solution as follows:

1. To 5 ml of distilled water add 3 drops of Reagent 1 and mix well.
2. Add 2 drops of Reagent 2 and mix well.
3. Add 2 drops of Reagent 3 and mix well.
4. Add 2 drops of the Hydrogen Peroxide Solution and mix well.

Incubate tissue sections with the substrate at room temperature until suitable staining develops. Development times should be determined by the investigator but generally 5 - 15 minutes provides good staining intensity. Longer incubations may increase sensitivity.

Wash the sections for 5 minutes in water.

The staining produced by the Vector® *NovaRED*™ Substrate Kit can be counterstained with Vector® Hematoxylin (Cat. No. H-3401) and Vector® Hematoxylin QS (Cat. No. H-3404). Sections should be dehydrated and permanently mounted in non-aqueous mounting media such as **VectaMount**™ (Cat. No. H-5000) using standard protocols. Do not use recycled alcohol with this product. (See reverse for counterstain compatibility chart.)

#### **Use in Membrane Staining**

For nitrocellulose or nylon membranes, the water volume may be increased to 15 ml in step 1 without a major reduction in sensitivity then follow instructions 1 through 4. Transfer membrane to a separate staining vessel *which has not been exposed to peroxidase* containing the substrate solution. Development time is generally 10 - 20 minutes at room temperature. When development is satisfactory, wash membrane in water for 5 minutes and air dry.

### **NOTES:**

We recommend using glass-distilled water in the preparation of the substrate. Deionized water may contain inhibitors of the peroxidase reaction.

The reagents should be stored at 4 °C and protected from light whenever possible. The color of the reagent solutions may darken with time. In general, this will have no effect on the quality or intensity of the staining.

Prolonged incubation in alcohol or use of alcohol-based differentiating solutions may decrease sensitivity.

For multiple labeling, Vector® *NovaRED*™ substrate provides excellent contrast with peroxidase substrates DAB/Ni and Vector® SG and with alkaline phosphatase substrates Vector® Blue and BCIP/NBT.

**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.