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## 4-CN SUBSTRATE KIT FOR PEROXIDASE

Catalog Number SK-4300

The 4-CN substrate kit contains all of the reagents necessary to prepare a working solution of 4-chloro-1-naphthol (4-CN) for staining nitrocellulose, nylon, or other membranes. 4-CN will yield a blue-black colored 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 Membrane Staining**

Immediately before use, prepare the substrate solution as follows.

1. To 5.0 ml of distilled water, add 2 drops of the Buffer Stock Solution and mix well.
2. Add 2 drops of the 4-CN Stock Solution and mix well.
3. Add 2 drops of the Hydrogen Peroxide Solution and mix well.

For nitrocellulose or nylon membranes, transfer membrane to a separate staining vessel *which has not been exposed to peroxidase*, containing a suitable volume of 4-CN substrate solution. Development time is generally 10 - 30 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 buffer. Deionized water may contain inhibitors of the peroxidase reaction.

The reagents should be stored at 4° C and protected from light whenever possible.

Blots developed with 4-CN should not be stored in direct contact with transparent adhesive tape or other plastic materials. Chemical bleaching of bands may occur with time.

**IMPORTANT:** Little is known about the toxicity and carcinogenicity of the substrate kit components. Care should be exercised when using these reagents including gloves, eye protection, lab coats, and good laboratory procedures. Dispose of the used working solution with care. Excess dilute working solutions may be discarded into a large volume of 3% potassium permanganate (KMnO<sub>4</sub>), 2% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) in deionized or distilled water. Dispose in accordance with local regulations.