

4CN PLUS  
For Chromogenic Detection of HRP  
Catalog Number NEL-300

**Description:** 4CN*Plus* is a modified, highly sensitive 4CN (4-chloro-1-naphthol) formulation designed for the chromogenic detection of horseradish peroxidase (HRP). The sensitivity of 4CN *Plus* is approximately 10X that of conventional formulations of 4CN and is equal or slightly better than diaminobenzidine (DAB).

**Components:**

4CN *Plus* Chromogenic Substrate: 15 mls in amber bottle

4CN *Plus* Substrate Diluent: 75 mls of concentrated (10X) buffer in white bottle

The amount of reagents supplied is enough to provide 750 mls of working substrate solution which is enough reagent for 3000 cm<sup>2</sup> of membrane. Use at least 0.25 mls of 4CN *Plus* per cm<sup>2</sup> of membrane.

Storage Conditions: Upon receipt, store the kit between 2-8°C.

Stability: The reagents perform best if used within a year from the date of purchase.

Preparation of Reagent:

For 10 mls of working solution:

1. Dilute 1 ml of 4CN *Plus* Substrate Diluent with 9 mls of distilled water.
2. Add 0.2 ml of 4CN *Plus* Chromogenic Substrate to the 10 mls of diluted Substrate Diluent and mix well. The solution might appear turbid; this is normal and does not interfere with substrate performance.

Usage of Reagent:

1. Add the prepared 4CN *Plus* substrate to a membrane(s) with immobilized HRP. Incubate at room temperature while shaking for 30 minutes. It is sometimes necessary or desirable to stop the reaction earlier if color development is rapid.
2. Terminate the reaction by washing the membrane(s) in distilled water. Air dry the membrane(s) and store.
3. For long term storage of the stained membrane(s), we have found that the type of plastic covering the membrane(s) is critical. We recommend the membrane(s) be placed in plastic sheet protectors (e.g. Boise Cascade Catalog No. L2-A8112).
4. Discard any unused working solution by disposal according to normal laboratory rules.

**Membrane Compatibility:** 4CN *Plus* has been formulated for use on Polyscreen™ PVDF Transfer Membrane, nitrocellulose membranes and the nylon membranes Gene Screen™ Hybridization Transfer Membrane and Gene Screen *Plus*® Hybridization Transfer Membrane.