



## Stability of 4 CN Peroxidase Substrate Solution

### Purpose:

To evaluate the stability of the 4 CN Peroxidase Substrate Solution stored at 4°C and room temperature over time.

### Reagents:

This study compares the performance of six lots of 4°CN Peroxidase Substrate Solution. Representative samples of each of the following lots were stored at 4°C and room temperature from the date of manufacturing.

| <u>4 CN Lot No.</u> | <u>Date of Mfg.</u> |
|---------------------|---------------------|
| EL11                | 12/83               |
| FC 03               | 3/84                |
| GD 31               | 4/85                |
| HA05                | 1/86                |
| JE01                | 5/87                |
| KF17                | 6/88                |

### Test Parameters:

The components are evaluated using a dot ELISA test procedure. The assays are performed on standard nitrocellulose (Schleicher & Schuell). The assay is performed as follows:

1. Set up dilution plate by performing 12 two-fold dilutions across a single row of a microtiter plate with Mouse IgG (Cappel), starting at a 0.1 mg/mL concentration in PBS.
2. Using an appropriate pen, mark nitrocellulose by making grid.
3. Wet nitrocellulose with reagent quality water.
4. From each well in the dilution plate, transfer 1.0 µl of the diluted Mouse IgG to appropriate spot on gridded membrane strips using a microdispenser. Allow strips to incubate for approximately 5 minutes to allow protein to adhere to the membrane.
5. Block strips with 1% BSA for 15 minutes at room temperature.
6. Incubate strips with Peroxidase-labeled Goat anti-Mouse IgG (H+L), Catalog No. 15-18-06 (Lot LC08-5) diluted 1:2500 in BSA Diluent/Blocking Solution (Catalog No.50-61-00) for 1 hour at room temperature.
7. Wash strips 3 times with 3 minutes soak periods using Wash Solution Concentrate (Catalog No. 50-63-00). After final wash, rinse strips with water.
8. Mix equal volumes of 4 CN Peroxidase Substrate Solution and Peroxidase Substrate Solution B to prepare a working substrate solution. Add 4 CN substrate. **NOTE:** A single lot of Peroxidase Substrate Solution B (Lot LE06) was used with all of the 4 CN substrates tested.
9. Stop substrate reaction after 10 minutes by rinsing nitrocellulose in water for 10-20 seconds.
10. Allow strips to air dry before storing.

### Results:

In this study, samples of 4 CN Peroxidase Substrate Solution, when stored at 4°C or room temperature, showed no significant variation in sensitivity between the lots. Precipitation was seen in all samples except for the 4°C sample of the latest lot KF17. This precipitation, which seems to increase as a function of time and temperature, did not significantly affect product performance.

### Conclusions:

KPL's 4 CN Peroxidase Substrate Solution appears very stable over time when stored at 4°C or room temperature for over 4 years. This study also demonstrates the exceptional consistency in product performance when stored at 4°C over an extended period of time.