

## — PROTOCOL —

## UltraScience Pico Plus/Pico Ultra/Femto Western Substrate Powder

### Catalog Number:

CCH321-P10L UltraScience Pico Plus Western Substrate Powder

CCH345-P10L UltraScience Pico Ultra Western Substrate Powder

CCH365-P10L UltraScience Femto Western Substrate Powder

**Storage:** Upon receipt make the ECL powder form into liquid form. Product is shipped at ambient temperature.

### Description

The **UltraScience Pico Plus/Pico Ultra/Femto Western Substrate Powder**, as a luminol-based enhanced chemiluminescent substrate, is sensitive and compatible with conducting immunoblots with horseradish peroxidase (HRP) – conjugated secondary antibodies. The low picogram to low femtogram detection of antigen is enabled by UltraScience Pico Plus-Pico Ultra/Femto Western Substrate's excellent sensitivity and long signal duration. Further, its long chemiluminescent signal duration makes both digital and film-based imaging possible without any loss of the signal. Appropriate primary and secondary antibody dilutions are suggested for attaining optimal signal intensity and duration.

- Ready-to-ship and significantly longer shelf life in the powder form!
- Significant reductions in transportation and storage costs, resulting in less carbon footprint!
- Empower custom production anywhere in the world!
- Significantly more sensitive than leading manufacturers on the market.

### Powder Dissolution

1. Prepare two 5L (liters) amber bottles and Deionized water (DI). One bottle is for Luminol solution (A), and the other is for Peroxide solution (B). Make sure the bottles are acid and alkali resistant.
2. Add the DI water into the bottles. Firstly, add 4.5 L of water, then proceed to open the bag (s) and completely pour all the powder out. Once, the powder has been completely added to the water, please keep adding water until it reaches 5 liters. Please ensure **Solution A is kept away from light**.
3. To properly combine the solutions, place them on a starter or Ultrasonic machine. Make sure the solutions have completely dissolved.
4. Finally, please transfer the well-combined solutions to your desired bottle size.

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**Chemiluminescent Development**

1. Keep the membrane moist in the wash buffer while preparing the substrate mixture.  
Please ensure the membrane does not dry out during the subsequent steps.
2. Mix Luminol solution and Peroxide Solution in a 1:1 ratio, and thoroughly agitate the chemiluminescent substrate solution well for preparing the 0.1 ml of solution / cm<sup>2</sup> of membrane.
  - For a mini-sized membrane (7 x 8.5 cm), 4 ml of solution is sufficient.
  - For a midi-sized membrane (8.5 x 13.5 cm), 10 ml of solution is sufficient.
3. Place the membrane with the protein side up on a clear and level surface or in a clean container.
4. Remove the membrane from the chemiluminescent substrate solution and drain off excessive solution.
5. Place the membrane in a plastic sheet protector or in plastic wrap to prevent the membrane from drying.
6. Image the membrane with a digital imager or by exposing to the X-ray film.

