— PROTOCOL —

UltraScence Pico Plus Western ECL Substrate

Catalog Number	Unit Size	Reactions
CCH321-B100ML	50ml x 2	Sufficient for over one mini-gel size membrane.
CCH321-004ML	2 ml x 2	Sufficient for over 25 mini-gel size membranes.

Storage : Store at 4℃ for 1 year

Description

The **UltraScence Pico Plus Western ECL Substrate**, as a luminol-based enhanced chemiluminescent substrate, is sensitive and compatible with conducting immunoblots with horseradish peroxidase (HRP) – conjugated secondary antibodies. The low picogram or high femtogram detection of antigen is enabled by UltraScence Pico Plus Western ECL 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.

- **No optimization required.** Switching to the UltraScence Pico Plus Western ECL Substrate from other brands, such as Pierce ECL and GE Healthcare, does not require optimization or protocol changes.
- **High degree of sensitivity and enhanced chemiluminescence duration.** UltraScence Pico Plus Western ECL Substrate enables an accurate low picogram or high femtogram detection of protein on the same immunoblot after a single exposure.
- **Optimized for use with PVDF and nitrocellulose membranes**.
- **Compatible with Western Blotting Markers.**
- **Optimized for film- and CCD-based imaging.**

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^2 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.

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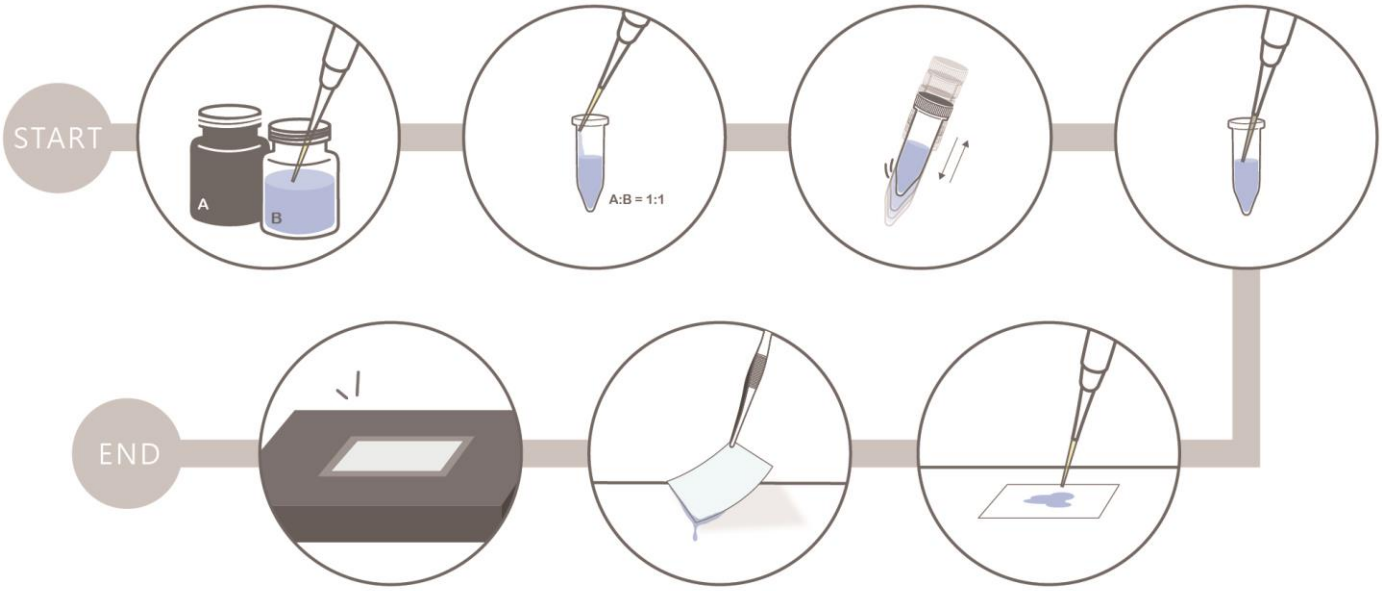

Troubleshooting

Problem	Cause	Solution
High Background	Overconcentrated primary or secondary antibody	*Decrease the antibody concentration.
		*Perform a dot blot to optimize the concentration.
	Insufficient wash	*Increase the frequency or duration.
	Incomplete blocking	*Decrease the antibody concentration.
		*Perform a dot blot to optimize the concentration.
No Reaction or Weak Signal	Insufficient antigen binding	*Decrease antibody concentration. *Optimize blocking reagents for achieving a balance between sensitivity and specificity.
	Poor antibody binding to the antigen	*Optimize detergent used for antibodies. *Increase the antibody incubation time.
No Reaction or Weak Signal	Proteins washed from the membrane during assay	*Reduce the number or intensity of wash
	Insufficient reagent volume	*Apply additional volumes of antibody blocking reagent, or wash solution.