



Cat. No. BCS-PR21001

BCodePro™ BrightSignal Femto ECL Solution

(Solution A 50 mL + Solution B 50 mL)

Application

Detection of HRP-conjugated antibodies on Western Blots using X-ray film or digital imaging systems

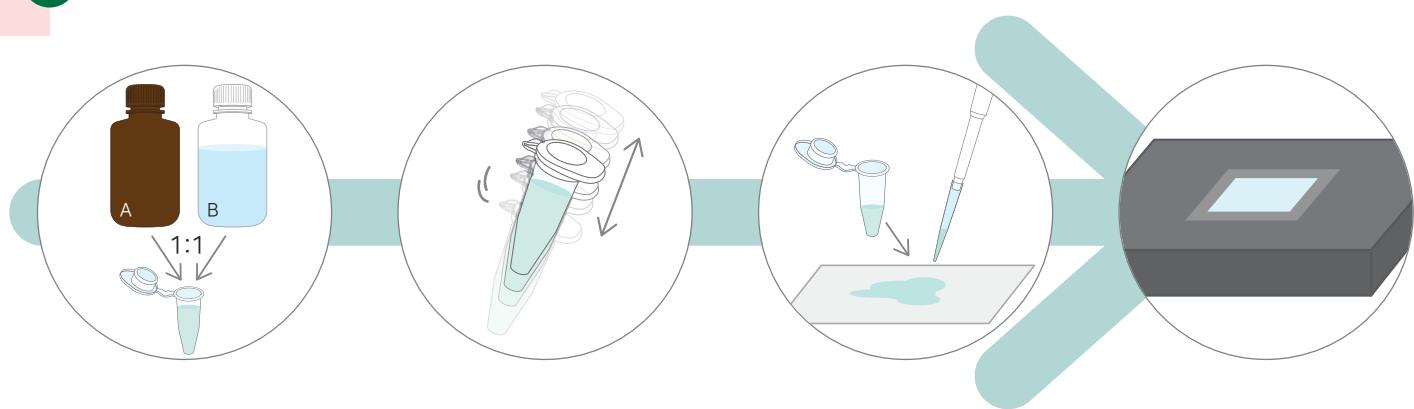
Storage

- Store at 4°C (Stable for up to 2 years)
- Be careful not to freeze

The BCodePro™ BrightSignal Femto ECL Solution, as a luminol-based enhanced chemiluminescent substrate, is sensitive and compatible with conducting immunoblots with horseradish peroxidase (HRP) – conjugated antibodies. The high femtogram to low femtogram detection of antigen is enabled by BCodePro™ BrightSignal Femto ECL Solution'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.

Protocol

- 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 Solution A and Solution B in a 1:1 ratio, and thoroughly agitate the chemiluminescent substrate solution well for preparing the 0.1 ml of solution / cm² 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 ECL solution and drain off excessive solution.
- 5 Place the membrane in a plastic sheet 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.



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
No reaction or weak signal	Insufficient antigen binding	Perform a dot blot to optimize the concentration
		Increase antibody concentration
	Poor antibody binding to the antigen	Optimize blocking reagents for achieving a balance between sensitivity and specificity
		Optimize detergent used for antibodies
	Proteins washed from the membrane during assay	Increase the antibody incubation time
	Insufficient reagent volume	Reduce the number or intensity of wash
		Apply additional volumes of antibody blocking reagent, or wash solution