DATASHEET

Ultra-Sense Femto-Plus Western ECL Substrate

Cat. No. 08X100003
Size: 2 x 50 mL (Substrate A + Substrate B)/Set
Revision Date: 2022-08

PRODUCT DESCRIPTION

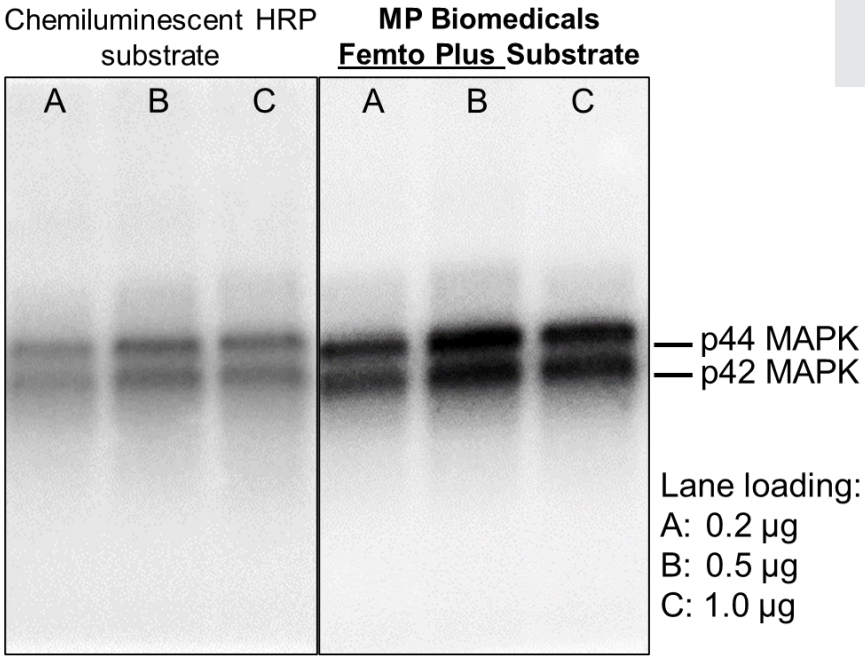
The MP Biomedicals Ultra-Sense Femto-Plus Western ECL Substrate, a luminol-based enhanced chemiluminescent substrate, is sensitive and compatible with conducting immunoblots with horseradish peroxidase (HRP) – conjugated secondary antibodies. The product is designed for the detection of target proteins in amounts that are too small to be detected with typical ECL substrates. The **low femtogram detection** (mid-zeptomole) of antigen is enabled by Ultra-Sense Femto-Plus Western ECL Substrate's excellent sensitivity and long signal duration. Furthermore, 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.

STORAGE CONDITIONS

Stable for up to 12 months at 4 °C.

FEATURES

- No protocol optimization required.
- Easy-to-Use, simply mix the solution A and B (1:1) for 30 seconds prior to use.
- High degree of sensitivity and enhanced chemiluminescence for femtogram detection of protein.
- Optimized for use with PVDF and nitrocellulose membranes.
- Compatible with Western Blotting Markers.
- Optimized for film- and CCD-based imaging.



Western blotting of HeLa cell lysates, using p44/42 MAPK rabbit monoclonal antibody (1 µg/mL) and secondary goat anti-rabbit HRP (1:5,000).

PROTOCOL

1. Keep the membrane moist in the wash buffer while preparing the substrate mixture (Solution A+B: 1:1 mixture). Ensure the membrane does not dry out in the next steps.
2. The chemiluminescent substrate solution is sufficient for the following membrane sizes:
   - For a small membrane (7 x 8 cm), 5 mL of solution is sufficient.
   - For a medium-sized membrane (8 x 13 cm), 10 mL of solution is sufficient.
3. Incubate the membrane (30 seconds, avoid light) with the substrate solution.
4. Remove the membrane from the chemiluminescent substrate solution and place it in a plastic sheet protector or plastic wrap to prevent the film from drying out.
5. Image the membrane with a digital imager or by exposure to X-ray film.

TROUBLESHOOTING

Problem	Cause	Solution
High Background	Too much primary or secondary antibody	Decrease the antibody concentration. Perform a dot blot to optimize the concentration.
	Insufficient wash	Increase the wash times or duration.
	Incomplete blocking	Increase blocking reagent concentration. Perform a dot blot to optimize the concentration.
No Reaction or Weak Signal	Insufficient antigen binding	Increase antibody concentration. Optimize blocking reagents for achieving a balance between sensitivity and specificity.
	Poor antibody binding to the antigen	Optimize detergent used for antibody binding. Increase the antibody incubation time.
	Proteins washed away from the membrane	Reduce the wash times.
	Insufficient antigen loading	Increase amounts of detected samples (such as whole cell lysate or immunoprecipitation elute).

MP BIOMEDICALS

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