MQ Western Substrate Ultra

Cat. No. WSU-M-100
Size: 100ml (50ml x 2)
Store at Room Temperature

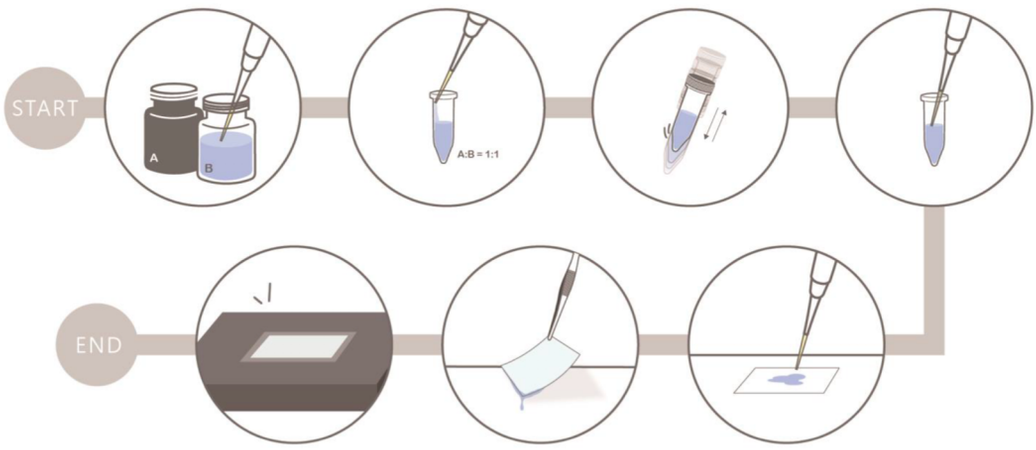
MOLEQULE-ON®

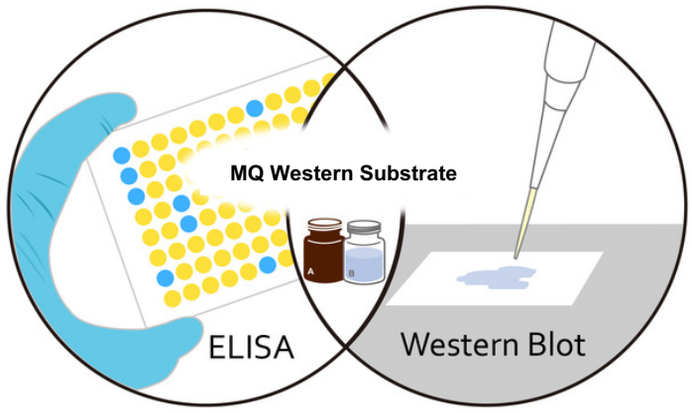
Description

The MQ Western Substrate Ultra works 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 mid femtogram detection of antigen is enabled by MQ Western Substrate Ultra shows 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.

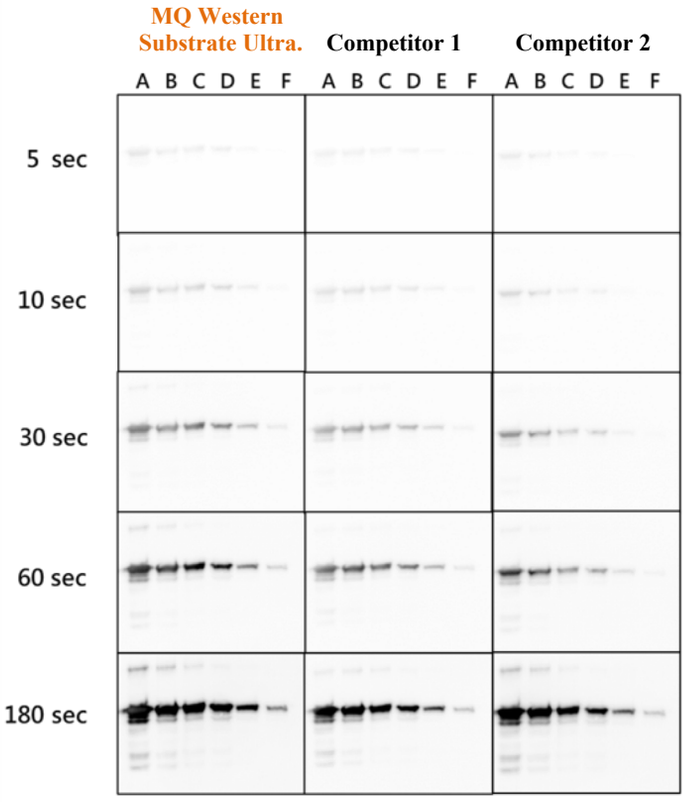
Features

- **No optimization required.** Switching to the MQ Western Substrate Ultra 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.** MQ Western Substrate Ultra 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.**





Low picogram to mid femtogram detection



MQ Western Substrate Ultra enables an accurate low picogram to mid femtogram detection of protein on the same immunoblot after a single exposure. Membranes were probed with GFP tag Rabbit PolyAb diluted at 1:10,000 of and then with Goat Anti-rabbit IgG/HRP secondary antibody (1:10,000) after serial dilution EGFP (Enhanced Green Fluorescent Protein) were prepared and applied in electrophoresis and protein transfer. Identical blots were incubated with the Western substrate. The blots were simultaneously exposed for 5 seconds, 10 seconds, 30 seconds, 60 seconds, and 180 seconds using imaging system.

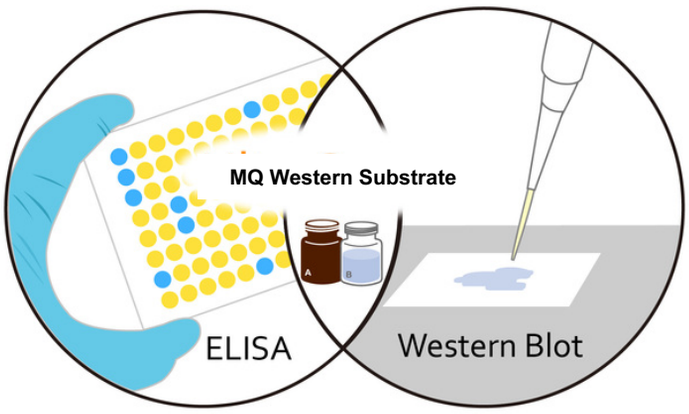
Protein conc.
Lane A: 1000 ng
Lane B: 500 ng
Lane C: 250 ng
Lane D: 100 ng
Lane E: 50 ng
Lane F: 25 ng

Procedure For 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.
    1. For a mini-sized membrane (7 x 8.5 cm), 4 ml of solution is sufficient.
    2. 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 MQ Western Substrate Plus 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.

	Advantages	Sensitivity
MQ Western Substrate Plus WSP-M-100	Best value for abundant protein detection and best sensitivity among entry-level western substrate	Low picogram to high femtogram
MQ Western Substrate Ultra WSU-M-100	Better choice when seeking low abundance proteins, over 30 times sensitivity than MQ Western Substrate Plus.	Low picogram to mid femtogram

MQ Western Substrate can also be applied as ELISA substrates.



Diluted capture antibody, 100 µL

↓ Seal and incubate overnight between 2-8 ℃

↓ Wash 4 times

Block with assay diluent, 400 µL

↓ Seal and incubate 1h, R.T., shake

↓ Wash 4 times

Mouse IL-2 standard (final conc. 0, 0.25, 1, 2.5, 10, 25, 50, 100 pg/mL) , 100 µL

↓ Seal and incubate 2h, R.T., shake

↓ Wash 4 times

Diluted detection antibody, 100 µL

↓ Seal and incubate 1h, R.T., shake

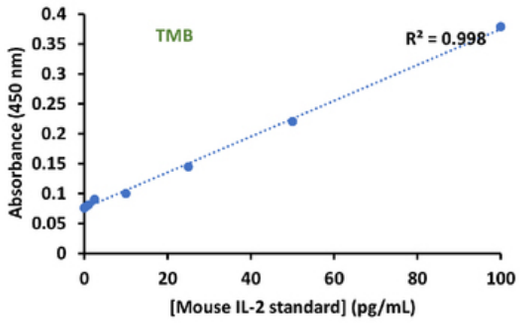
↓ Wash 4 times

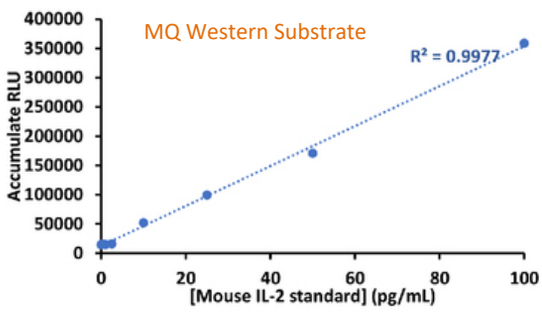
Diluted avidin-HRP, 100 µL

↓ Seal and incubate 30 min, R.T., shake

↓ Wash 5 times

TMB substrate solution, 100 µL	MQ Western Substrate, 200ul
↓ Incubate in dark, 30 min	↓
Stop solution, 100 µL	Luminescent detection for 1h
↓	
Absorbance 450 nm	





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.