## Ultra-sensitive ECL Chemiluminescent Substrate

### Catalogue Information

| Catalogue No. | Product Name | Pack Size |
|---|---|---|
| WS BL520A | Ultra-sensitive ECL Chemiluminescent Substrate | 100 mL |
| WS BL520B | Ultra-sensitive ECL Chemiluminescent Substrate | 500 mL |

### Product Description

The Ultra-sensitive ECL Chemiluminescent Substrate provides bright signal output and picogram-level detection sensitivity for Western blot assays utilizing horseradish peroxidase (HRP)-conjugated antibodies. It is compatible with various membranes, blocking reagents, and antibody diluents, offering superior performance, versatility, and cost-effectiveness for a wide range of immunoblotting applications.

### Product Components

| Catalogue No. | Component | Volume |
|---|---|---|
| BL520A-1 / BL520B-1 | Enhanced Luminescent Solution A | 50 mL / 250 mL |
| BL520A-2 / BL520B-2 | Stabilizing Solution B | 50 mL / 250 mL |

### Application

For use in HRP-based Western blotting and nucleic acid hybridization assays.

1. **High Sensitivity**: Detects protein bands at the low picogram level on nitrocellulose or PVDF membranes.
2. **Long-lasting Signal**: Under optimal conditions, the signal remains visible for 6–8 hours after substrate incubation.
3. **Stable Reagents**: Working solution remains stable for up to 24 hours after preparation.
4. **Flexible Detection Methods**: Suitable for X-ray film, CCD, or laser imaging systems.
5. **Cost-effective**: Optimized for diluted antibody concentrations:
    - Primary antibody: 0.2–1.0 μg/mL (1:1,000–1:5,000 dilution from 1 mg/mL stock)
    - Secondary antibody: 10–50 ng/mL (1:20,000–1:100,000 dilution from 1 mg/mL stock)
6. **Simple and Compatible**: Can replace other commercial ECL substrates without requiring workflow modification.

### Usage Instructions

1. Perform standard SDS-PAGE, transfer, and Western blot procedures using HRP-conjugated antibodies or biotin-streptavidin-HRP systems.
2. During the final wash step, prepare the ECL working solution by mixing equal volumes of Solution A and Solution B in a clean container. Use separate pipette tips for each solution to prevent contamination. The working solution should be used immediately for optimal sensitivity; if kept at room temperature for several hours, sensitivity may slightly decrease.
3. After the final wash, use tweezers to place the membrane on clean cling film or a plastic tray, removing excess liquid with filter paper. Avoid touching the protein side of the membrane.
4. Add sufficient prepared ECL working solution to cover the membrane evenly. Incubate for 1–3 minutes.
5. Remove excess liquid with filter paper, sandwich the membrane between cling films, and detect signal using X-ray film or a chemiluminescent imager.
6. **X-ray Film Detection**: Place the membrane in a cassette with the protein side facing up. Expose the film in a darkroom for various times (from seconds to minutes), then develop and fix.
7. **Chemiluminescent Imager Detection**: Place the membrane in the imager and follow instrument-specific instructions. Initial exposure for 60 seconds is recommended; adjust exposure time as needed for optimal results

**The chemiluminescent signal is strongest within 5–30 minutes after substrate incubation and can persist for several hours, though intensity will gradually decrease over time.**

**Note:**

1. Optimize system parameters such as sample loading, antibody concentrations, and membrane type for best results.
2. No single blocking reagent is optimal for all systems; select a blocking buffer suitable for your assay.
3. Avoid using milk-based blockers in biotin/streptavidin systems as endogenous biotin may cause high background.
4. Do not allow membranes to dry during the experiment.
5. Sodium azide inhibits HRP; do not use as a preservative in buffers.
6. Handle membranes with gloves or clean tweezers to avoid contamination.
7. Short-term exposure to ambient light will not damage the reagents, but avoid prolonged exposure to strong light sources.

**Storage Conditions**

Transport at room temperature. Store at 4 °C, tightly sealed and protected from light. Stable for one year under recommended storage conditions. For short-term use, reagents can be kept at room temperature.

**Troubleshooting**

| | |
|---|---|
| Problem: | Film shows reversed or dark bands |
| Cause: | Excess HRP in the system. |
| Solution: | Dilute HRP-conjugated secondary antibody at least 10-fold. |
| Problem: | Brown or yellow bands appear on the membrane / signal duration < 8 h / weak or no signal |
| Cause: | Excess HRP depletes substrate rapidly. |
| Solution: | Dilute HRP-conjugated secondary antibody at least 10-fold. |
| Cause: | Insufficient antigen or antibody. |
| Solution: | Increase amount of antigen or antibody. |
| Cause: | Poor transfer efficiency or low HRP/substrate activity. |
| Solution: | Optimize transfer and verify reagent activity. |
| Problem: | High background |
| Cause: | Excess HRP or incomplete blocking. |
| Solution: | Dilute HRP antibody, optimize blocking buffer, or increase wash time. |
| Cause: | Overexposure of film. |
| Solution: | Shorten exposure or use background reduction filters. |
| Problem: | Spotty bands or uneven signal |
| Cause: | Uneven membrane hydration or air bubbles. |
| Solution: | Rehydrate membrane properly and remove air bubbles before exposure. |
| Cause: | HRP aggregates in secondary antibody. |
| Solution: | Filter secondary antibody through a 0.2 μm filter before use. |
| Problem: | Non-specific bands |
| Cause: | Excess HRP or SDS-induced non-specific binding. |
| Solution: | Reduce HRP concentration and avoid SDS during detection. |

**Notice**

For in vitro research use only, not for diagnostic or therapeutic use. This product is not a medical device.
Contact:

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