

### HiSensiFluor™ ECL substrate (FemtoPlus, for Western blotting)

<u>Product Name</u>	<u>Product Code</u>	<u>Kit Packing</u>
HiSensiFluor™ ECL substrate	ML259-50ML	50ML
(FemtoPlus, for Western blotting)	ML259-100ML	100ML

#### Introduction:

Western blotting, also known as protein immunoblotting, is a highly sensitive analytical technique used to detect specific proteins within complex mixtures. This method leverages the principle of antigen-antibody interaction, employing monoclonal or polyclonal antibodies to identify target proteins.

HiSensiFluor™ ECL substrate (FemtoPlus, for Western blotting) is a luminol-based enhanced chemiluminescent substrate employed for the detection of proteins on Western blots via chemiluminescence.

#### Description:

Western blotting, a standard protein analysis technique first described by Towbin in 1979, typically involves electrophoretic transfer of proteins to a membrane followed by antibody-based detection. The proteins transferred on to the blot after immunodetection can be visualized by colorimetric, chemiluminescence, and fluorescence techniques.

HiSensiFluor™ ECL substrate (FemtoPlus, for Western blotting) is a chemiluminescent reagent designed for sensitive protein visualization in Western blots. It is specifically compatible with horseradish peroxidase (HRP)-conjugated secondary antibodies.

#### Features:

- Ready to use solution.
- **High Sensitivity:** Enables the detection of proteins in the low picogram to mid-femtogram range.
- **Long Signal Duration:** Allows for both film-based and digital imaging without signal loss.
- **Versatile Compatibility:** Suitable for use with both PVDF and nitrocellulose membranes.
- **Marker Compatibility:** Compatible with common Western Blotting Markers.
- **Imaging Flexibility:** Compatible with both film and CCD-based imaging systems.

**Contents:**

HiSensiFluor™ ECL substrate (FemtoPlus, for Western blotting) contains following components:

Product Code	Reagents	Quantity	
		50ML	100ML
DS1917	Luminol Solution	25 ml	50 ml
DS1918	Peroxide Solution	25 ml	50 ml

**Intended Use:**

Recommended for protein Immunodetection procedures.

**Note:** Appropriate primary and secondary antibody dilutions are suggested for attaining optimal signal intensity and duration.

**Storage:**

Store the HiSensiFluor™ ECL substrate (FemtoPlus, for Western blotting) between 15-25°C. Under recommended condition, the solutions are stable for 6 months.

**General Preparation Instructions:**

1. Read the procedure carefully before starting the experiment.
2. Combine Luminol solution and Peroxide Solution in equal volumes (1:1 ratio) and mix the solution thoroughly.
3. It is recommended to use 4 mL of solution for mini-sized membranes (7 x 8.5 cm) and 10 mL for midi-sized membranes (8.5 x 13.5 cm). This volume provides 0.1 mL of solution per square centimeter of membrane. Appropriate volume of the substrate to be added can be optimized as per the requirement.
4. Keep the membrane consistently moist with wash buffer during substrate preparation and subsequent steps.

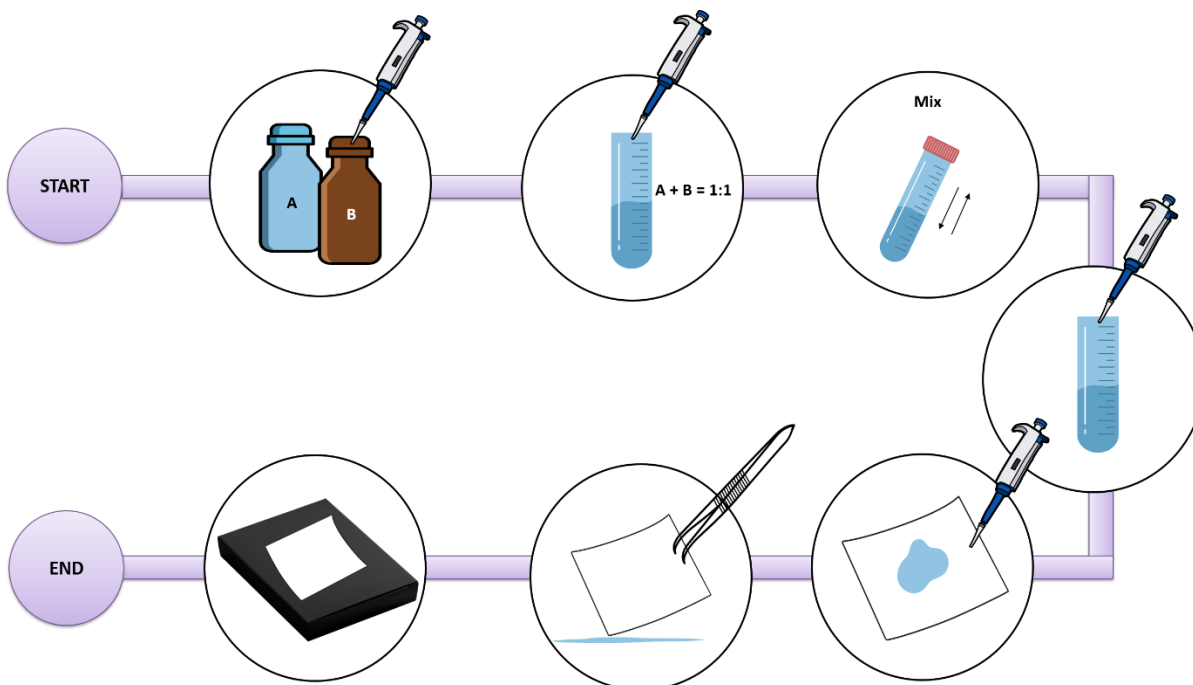
**Important Note:** This product cannot be used under direct light. It is to be used under dark conditions.

**Procedure:**

1. Place the membrane with the protein side facing upward on a flat, clean surface or within a clean container.
2. Pour the freshly mixed working solution onto the membrane containing the HRP-conjugated antibodies.
3. Incubate the membrane for a specified time depending on the desired signal intensity.

4. Remove the membrane from the chemiluminescent substrate solution and drain off excessive solution.
5. Enclose the membrane in a plastic sheet protector or wrap it tightly with plastic film to prevent it from drying out.
6. Capture an image of the membrane using a digital imager. Alternatively, expose the membrane to X-ray film for image development.

**Flowchart:**



**Fig 1: Detection steps involved in immunodetection using HiSensiFluor™ ECL substrate (FemtoPlus, for Western blotting)**

**Warning and Precautions**

Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good laboratory practices while handling samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

**Limitations**

Development of protein bands depends upon the protein samples taken for detection, detection time and the concentration of antibodies.

## Performance and Evaluation

Performance of the product is expected when the product is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.









## Safety Information

HiSensiFluor™ ECL substrate (FemtoPlus, for Western blotting) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

## Technical Assistance

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at [mb@himedialabs.com](mailto:mb@himedialabs.com).

## Symbol

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIML259

Rev No.: 00

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### Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

HiMedia Laboratories Pvt. Ltd. Reg.office : Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane, (West) 400604, Maharashtra, INDIA.  
Customer Care No.: 00-91-22-6116 9797 Tel: 00-91-22-6147 1919, 6903 4800 Email: [techhelp@himedialabs.com](mailto:techhelp@himedialabs.com) Website: [www.himedialabs.com](http://www.himedialabs.com)