

LumiFlash™ Femto Chemiluminescent Substrate

LF24-100

V1.0

Store at 2-8 °C
For Research Use Only

■ Introduction

LumiFlash™ Femto Chemiluminescent Substrate is the most sensitive chemiluminescent reagent in our **LumiFlash™** Series products. It is an extremely sensitive ECL (enhanced chemiluminescent substrate) for detection of low-femtogram level protein with horseradish peroxidase (HRP) in Western blotting. **LumiFlash™ Femto Chemiluminescent Substrate** provides high intense signal output resulting a brighter background on both PVDF and nitrocellulose membrane. It fulfills the need for detection of target proteins in trace amounts which are too small to be detected with general ECL substrate.

■ Product Components

LumiFlash™ Femto Chemiluminescent Substrate (LF24-100)

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|--------------------------------|-------|----------|
| Solution A (Luminol Solution) | 50 mL | 1 bottle |
| Solution B (Peroxide Solution) | 50 mL | 1 bottle |
| User's manual | | |

■ Safety Information

Please wear gloves, lab coat and goggles while operating. Prevent contact product directly. In case of contacting, wash with large amount of water.

■ Storage

LumiFlash™ Femto Chemiluminescent Substrate should be stored at 2-8 °C and shielded from light. Expiration date is labeled on the bottle or box.

■ Materials needed but not provided

1. PVDF or nitrocellulose membrane
2. Wash buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05–0.1% Tween®-20
PBS: 10 mM KH₂PO₄, 150 mM NaCl, pH 7.4
TBS: 25 mM Tris, 150 mM NaCl, pH 7.4
3. Blocking buffer: 1–5% (w/v) blocking agent (e.g., casein, BSA, or gelatin) in wash buffer
4. Specific primary antibody for interested protein, diluted in blocking buffer
5. HRP-conjugated secondary antibody, specific for primary antibody, diluted in blocking buffer
6. X-ray film or chemiluminescence image acquisition systems

■ Instruction

A. Protein transfer

1. Perform 1D or 2D electrophoresis for protein separation.
2. Move the electrophoretic gel into appropriate transfer buffer and equilibrate for 10 minutes.
3. Wet the PVDF or nitrocellulose membrane in transfer buffer. (For PVDF membrane, it is necessary to pre-wet it in methanol before moving into transfer buffer).
4. Assemble the transferring sandwich as the order of two filter papers, gel, membrane and two filter papers.
5. Transfer proteins according to blotting apparatus manufacturer's instruction.

B. Antibody incubation

1. Add BSA, skim milk based blocking buffer or **BlockPRO™ Blocking Buffer (BP01-1L)** and incubate at room temperature for 30 minutes.
2. Prepare the primary antibody by diluting it with blocking buffer according to the manufacturer's instruction or previous experience.

NOTE: Due to the good sensitivity of chemiluminescent detection, primary antibody dilution factor can be increased 5-10 folds for optimal signal to noise ratio.

3. Add primary antibody and incubate at room temperature for at least 1 hour with gentle agitation. For more specific interaction between primary and antigen proteins, it is recommended to perform additional incubation at 4 °C for 8-12 hours.

B. Antibody incubation (~continued)

4. Decant the primary antibody solution thoroughly. Wash the membrane at least three times with ample amount of fresh Wash buffer for 10 minutes.
5. Prepare the secondary antibody by diluting it with blocking buffer according to the manufacturer's instruction.

NOTE: Due to the good sensitivity of chemiluminescent detection, the suggested dilution range of secondary antibody is 1:10,000-1:500,000.

6. Add secondary antibody and incubate at room temperature for 1 hour with gentle agitation.
7. Decant the secondary antibody solution thoroughly. Wash the membrane of at least four times with ample amount of fresh Wash buffer for 10 minutes.

C. Chemiluminescent detection

1. To prepare working HRP substrate, mix equal volume of Solution A and Solution B in a clean tube freshly. 0.1 mL of working HRP substrate is sufficient per 1 cm² membrane area.

NOTE: With proper light shielding, the HRP working substrate can stand at room temperature for 3 minutes and provide more stable signal.

2. In the dark room or box, place the membrane side up in a clean box or plastic wrap. Add HRP working substrate onto the membrane.
3. Incubate the membrane at room temperature for 30 seconds.
4. Overlay plastic wrap or a transparency sheet on the wet membrane.

NOTE: Do not use filter papers to overdrain the HRP substrate. It will decrease the signal significantly. Keep the membrane wet while exposing!! (see next step)

5. Expose the membrane to appropriate X-ray film or by chemiluminescence image acquisition system. It is recommended to use 30 seconds as the initial exposure time.

D. Stripping of PVDF membrane

1. Incubate membrane in stripping buffer (62.5 mM Tris-HCl pH 6.8, 100 mM β -mercaptoethanol and 2% (w/v) SDS) for 30 minutes at 50-70 °C.
2. Wash the membrane twice in Wash buffer for 10 minutes each.
3. To ensure complete removal of antibodies, incubate the membrane with LumiFlash™ HRP working substrate and expose against X-ray film for 5 minutes. No signal should be observed for complete stripping.

■ Troubleshooting

| Problem | Possible cause | Remedy |
|--------------------------|---|---|
| No signal or weak signal | Poor transfer efficiency | Optimize the membrane transferring procedure |
| | Insufficient antigen | Increase the amount of loaded antigen Make sure the blot have been store correctly to avoid the degradation of target protein |
| | The concentration of primary and secondary antibody is too low | Increase the concentration of the primary and/or the secondary antibody |
| | Inappropriate storage/preparation of the ECL detection reagents | Use HRP or HRP conjugates to check the applicability of ECL reagents |
| | Too short exposure time | Extend exposure time |
| Excessive signal | Antigen or antibody excess | Reduce the amount of loaded antigen Dilute the primary antibody and/or the secondary antibody |
| | Antigen or antibody excess | Optimize the condition by reducing the amount of antigen, or the concentration of the primary antibody and/or secondary antibody. Initially, reduce the secondary antibody to 20% of the original usage |
| | Inappropriate blocking | Try different blocking substrate such as gelatin, casein, skim milk or casein |
| High Background | Inadequate washing | Increase the concentration of Tween-20 in washing solution Increase the washing steps between the hybridization procedures Extend washing time |
| | | Overexposure to film |

■ Related Visual Protein Products

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| BlockPRO™ Blocking Buffer | BP01-1L | 1 L |
| BlockPRO™ Protein-Free Blocking Buffer | BF01-1L | 1 L |
| BlockPRO™ 1 Min Protein-Free Blocking Buffer | BM01-1L | 1 L |
| LumiFlash™ Prime Chemiluminescent Substrate, HRP System | LF01-500 | 500 mL |
| LumiFlash™ Ultima Chemiluminescent Substrate, HRP System | LF08-500 | 500 mL |
| LumiFlash™ Infinity Chemiluminescent Substrate, HRP System | LF16-500 | 500 mL |
| LuminolPen™, HRP System | LH03-50 | 1 pen |
| LuminolPen™ EZ, HRP System | LH05-50 | 1 pen |