# LumiFlash™ Ultima Chemiluminescent Substrate, HRP

1R7250

## Introduction

**LumiFlash<sup>TM</sup> Ultima Chemiluminescent Substrate, HRP** is an advanced ECL product in terms of efficacy and sensitivity for chemiluminescent detection of immobilized proteins (Western blotting), conjugated with Horseradish Peroxidase (HRP) directly or indirectly. **LumiFlash<sup>TM</sup> Ultima Chemiluminescent Substrate, HRP** provides high sensitivity in Western blotting application. It just needs very short exposure time to the X-ray film or in other documentation systems, and can get low background and high signal in clear images. **LumiFlash<sup>TM</sup> Ultima Chemiluminescent Substrate, HRP** is the perfect choice for most Western blotting applications.

| | |
|---|---|
| **Duration** | >6 hours |
| **Detection Method** | X-ray film or imaging acquisition system |
| **Typical Antibody dilution** | Primary: 1:1,000–1:20,000 |
| | Secondary: 1:40,000–1:200,000 |
| **Shelf life** | ≥1 year at time of receipt |
| **Recommended Initial Exposure** | Time 1 minute (to X ray film) |

## Product Components

**LumiFlash<sup>TM</sup> Ultima Chemiluminescent Substrate, HRP  (1R7250)**

| | | |
|---|---|---|
| Solution A (Substrate Solution) | 250mL | 1 bottle |
| Solution B (Peroxide Solution) | 250mL | 1 bottle |
| User's manual | | 1 booklet |

## 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<sup>TM</sup> Ultima Chemiluminescent Substrate, HRP** should be **stored at 4 <sup>O</sup>C and shielded from light**. Please use up the product in in 1 year.

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# 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 sodium phosphate, 150 mM NaCl, pH 7.2
   TBS: 10 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. A 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.
6. Wash the transferred membrane of three times by Wash buffer for 5 minutes.

## B. Antibody incubation

1. Add BSA, skim milk based blocking buffer or **BlockPRO™ Protein-Free Blocking buffer (1R5100)** 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. **(Due to the good sensitivity of chemiluminenscent detection, previous antibody dilution factor can be increased 2-5 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 $^O$C 8-12 hours.
4. Decant the primary antibody solution thoroughly. Wash the membrane of at least three times with ample amount of fresh Wash buffer for 10 minutes.

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5. Prepare the secondary antibody by diluting it with Blocking buffer according to the manufacturer's instruction. **(Due to the good sensitivity of chemiluminenscent detection, secondary antibody dilution can start from 1:20,000)**

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. Chemiluminenscent 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 cm$^2$ membrane area.

2. Allow the HRP working substrate to stand at room temperature for 3 minutes with proper light shielding.

3. 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.

4. Incubate the membrane at room temperature for 10 seconds.

5. 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)

6. Expose the membrane to appropriate X-ray film or by chemiluminenscent image acquisition system. It is recommended to use 1 minute as the initial exposure time.

## D. Striping of PVDF membrane

The immunoblot of PVDF membrane can be stripped of antibodies, and then reprobed.

1. Incubate membrane in stripping buffer (62.5mM Tris-HCl pH 6.8, 100mM β-mercaptoethanol and 2% (w/v) SDS) for 30 minutes at 50-70 $^O$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 *Ultima* HRP working substrate and expose against X-ray film for 5 minutes. No signal should be observed for complete stripping.

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# Trouble Shooting

| 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. |
| **High Background** | 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. |
| | Inadequate washing | Increase the concentration of Tween-20 in washing solution. Increase the washing steps between the hybridization procedures.\n\nExtend washing time. |
| | Overexposure to film | Shorten the exposure time. |

# Related Products

| | | |
|---|---|---|
| **LumiFlash Prime Chemiluminescent Substrate, HRP** | **1R7240** | **500mL** |
| **LumiFlash Infinity Chemiluminescent Substrate, HRP** | **1R5091** | **500mL** |
| **BlockPRO Protein-Free Blocking Buffer** | **1R5100** | **1000mL** |
| **LuminolPen, HRP System** | **1R7260** | **1 pen** |
| **LuminolPen Lite, HRP System** | **1R7270** | **1 pen** |

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