Owi ECL RevelBIOt® Femto

Chemiluminescent substrate with persistent signal
User Manual

Reference: 258498

For 1000 cm2 membrane (2x 50 ml)
Storage and stability: **one year at room temperature**

FOR RESEARCH ONLY

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SECTION 1: DESCRIPTION

Owi ECL RevelBIOt® Femto is a chemiluminescent substrate of HRP with a very intense signal that is persistent (24h). It allows the detection of antigens immobilized on a membrane (Western blot). The very intense signal makes it possible to obtain a sensitivity threshold lower than the picogram.

SECTION 2: PRECAUTIONS

- In order to obtain the best signal-to-noise ratio, it is recommended to:
  - Optimize primary and secondary antibody concentrations.
  - To test several blockers and empirically determine which one is most suitable.
  - Add Tween-20® to antibody blocking and dilution buffers to reduce non-specific signals.

Throughout the protocol, the membrane should never dry out. To do this, make sure you use the right amount of buffers, whether it is the blocking buffer, the wash buffer, the diluted antibody or **the active solution Owi ECL RevelBIOt® Femto**.

- Incubation is recommended on a stirring platform or equivalent system.
- When handling detection films or reagents, wear powder-free gloves or use forceps.
- Do not use sodium azide as a buffer preservative as it is an HRP inhibitor.
- Use clean utensils. Rust marks on scissors, pliers can cause a high background noise.
- **Owi ECL RevelBIOt® Femto** active Solution is stable for 24 hours at room temperature. If it is not used extemporaneously, store it in an opaque bottle.

SECTION 3: COMPONENTS

	Solution A - Brown Bottle	Solution B - Clear Bottle
OZYB002-1000 For 1000 cm2 of membrane	50 ml Luminol/Enhancer	50 ml tampon Péroxide

SECTION 4: WORKING SOLUTIONS

- **Tampon de dilution:** Tris-Buffered Saline (TBS)
- **Wash Buffer:** TBS-Tween 0.1%
- **Blocking buffer:** dissolve the blocking agent (non-fat milk powder, BSA, etc.) in TBS-Tween 0.1%
- **Primary antibody:** Prepare a stock solution of 1 mg/mL of the antigen-specific antibody to be detected in the dilution buffer. To obtain the ready-to-use solution, dilute this stock solution in the blocking buffer according to the manufacturer's dilution recommendations. In standard conditions, the dilution is between 1:1000 and 1:50,000 or 20 ng/ml to 1 µg/ml.
- **HRP conjugated secondary antibody:** To obtain the ready-to-use solution, dilute it in the blocking buffer. Follow the manufacturer's recommendations to optimize dilution. Under standard conditions, for an HRP conjugated antibody at 1 mg/ml, the dilution is between 1:50,000 and 1:250,000 or 4 to 20 ng/ml.

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- **Owi ECL RevelBIOt® Femto active Solution:** it is obtained by mixing in equal quantities (50/50) the A solution (Luminol/Enhancer, contained in the brown bottle) and the B solution (Peroxide buffer, contained in the clear bottle).

SECTION 5: MATERIAL REQUIRED

- Protein electrophoresis tanks, Western blot membrane transfer apparatus.
- Nitrocellulose (or PVDF) membrane for Western blot application.
- Stirring platform or equivalent system.
- Detection:
  - Imager with a CCD camera compatible with weak signal detection and configured for Western blotting.
    or
  - X-ray sensitive film, cassette and fixation and development reagents dedicated to autoradiography.

SECTION 6: QUICK-PROTOCOL

STEP 1	STEP 2	STEP 3
Protein separation by electrophoresis	Transfer to membrane	Blocking non-specific sites
STEP 4	**STEP 5**	**STEP 6**
Incubation with the primary antibody specific to the antigen to be detected	First Membrane Wash	Membrane Incubation with HRP-Conjugated Secondary Antibody
STEP 7	**STEP 8**	**STEP 9**
Second Membrane Wash	Incubation 5 minutes with **Owi ECL RevelBIOt® Femto active solution** (50/50 solution A and solution B)	Exposure of the membrane to X-ray sensitive film or use of an imager configured for Western Blot

SECTION 7: COMPLETE WESTERN BLOT PROTOCOL

Step 1 – Separate the protein carrying the antigen of interest by electrophoresis.

Step 2 – Transfer the proteins to a nitrocellulose membrane (a PVDF membrane or other type can be used; where appropriate, optimizations may be required).

Step 3 – Incubate the membrane in the blocking buffer for 1 hour at room temperature on a stirring platform or equivalent system. Alternatively, the membrane can be incubated always in the blocking buffer overnight, between +2°C and +8°C.

Step 4 – Remove the blocking pad. Incubate the membrane with the diluted antibody for 1 hour on a stirring platform or equivalent system. Follow the dilutions recommended in the "Work Solutions" section.

Step 5 – Wash the membrane in the wash pad on a stirring platform or equivalent system by doing at least 4 to 6 washes of 5 minutes or more each. Each wash is carried out at room temperature with fresh wash pad. Increasing the buffer volume and the number of washes reduces the background noise level.

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Step 6 – Flush the membrane and incubate on a stirring platform or equivalent system for one hour with the diluted HRP conjugated secondary antibody. Follow the dilutions recommended in the "Work Solutions" section.

Step 7 – Wash the membrane (see step 5).

Step 8 – Incubate the membrane in **the active Owi ECL RevelBIOt® Femto** solution (see section "Working Solutions") for 5 minutes. The total volume must cover the membrane; under standard conditions, use 0.1 ml of **Owi ECL RevelBIOt® Femto** active solution per cm2 of membrane. **Owi ECL RevelBIOt® Femto** Active Solution is stable for 24 hours at room temperature. Incubation can be done in daylight or in a normally lit room, but not near bright light. If **Owi ECL RevelBIOt® Femto** Active Solution is not used extemporaneously, protect it from light by storing it in an opaque bottle.

Step 9 – Remove the membrane from the Owi ECL RevelBIOt® Femto **Active Solution**

- Drain excess detection reagent by holding the membrane vertically and putting one edge of the membrane in contact with a paper towel.
- Wrap the membrane in extra-thin transparent plastic film, such as "Saran Wrap" or equivalent. Be careful to remove excess liquid and air bubbles. Reminder: the membrane should never dry out (see "Precautions" section).
- Revelation by autoradiography on film:
    - Place the protected membrane in an X-ray film cassette, protein side up. The X-ray sensitive film should remain dry during exposure. Make sure that the wet membrane is never in contact with the film.
    - Turn off the light, place the film on top of the membrane, close the cassette. Do not move the film during the exposure to avoid any artifacts.
    - Expose for a minute. Exposure time may vary to optimize results.
    - Remove the film and immediately place a new, unexposed film. Close the cassette. The light emission continues for 24 hours, decreasing over time. It is therefore possible if necessary to redo one or more other exhibitions with new films. However, the half-life of the HRP enzyme is such that longer exposure times may be required.
    - Develop the film with the appropriate fixation and development reagents.
- Imaging Revelation: The device with a CCD camera must be configured for Western blotting.
    - The exposure time varies from machine to machine and can be longer than with autoradiographic film.

Note: at the end of the protocol, you can store the membrane wrapped in the transparent film "Saran Wrap" at a temperature of +2 to +8°C. The membrane can be dehybridized and rehybridized multiple times.

SECTION 8: DEHYBRIDIZATION/REHYBRIDIZATION PROTOCOL

Step 1 – Immerse the membrane in the dehybridization buffer (2-Mercaptoethanol 100 mM, SDS 2%, Tris-HCl 62.5 mM, pH6.7) and incubate for 30 minutes at 50°C. If you want to work in more stringent conditions, incubation can be done at 70°C for a period of more than 30 minutes.

Step 2 – Incubate the membrane for 2 x 10 minutes at room temperature in PBS-Tween or TBS-Tween buffer. Note: Following this step, the membrane can be incubated with the active Owi ECL RevelBIOt® Femto solution and then exposed on film or in a CCD imager to check for any signal.

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Step 3 – Incubate the membrane in the blocking buffer (see section "Work Solutions") for 1 hour at room temperature.

Step 4 – Repeat the Western blot protocol in Step 4.

SECTION 9: TROUBLESHOOTING

9.1 High Background

Possible causes	Notes / Solutions
Antibody concentrations that are too high	• Dilute primary and/or secondary antibody concentrations
Inoperative blocking agent	• Verify that the blocking buffer has been prepared correctly • Ensure that the blocking agent is present in diluted solutions of primary and secondary antibodies • Change the blocking agent: 1 to 10% BSA to TBS-Tween or PBS-Tween (prepared extemporaneously); 0.5% to 3% gelatin in TBS-Tween or PBS-Tween (prepared extemporaneously)
Inefficient washing	• Increase wash time and wash buffer volume • Add Tween (if you haven't already) • Increase the concentration of Tween in the wash pad
Contaminated Tampons	• Make sure all buffers are prepared extemporaneously and filtered
Contamination of equipment (tanks/transfer device).	• Clean or replace devices
Membrane Problems	• Check that the membrane is completely submerged and remains well moistened in all solutions • Use good quality nitrocellulose (or PVDF) membranes • A damaged membrane can cause non-specific antibody binding. Handle the membrane with care using powder-free gloves (see section "Precautions") • Use clean tongs to handle the membrane after washing
Detection Solution **Owi ECL RevelBlOt® Femto**	• Excess Owi ECL RevelBlOt® Femto ^{Active} **Solution.** Remove excess well with paper towels before placing the membrane in the cassette
Overexposure (film or imager)	• Expose the film for a shorter time (15 seconds may be enough). If the exposure time is too short for easy handling, reduce the antibody concentration • Leave the membrane in the dark before exposing again. • With an imager configured for Western blotting, it is possible to use a short exposure time and then accumulate the signal in series

9.2 Weak signal, no signal

Possible causes	Notes / Solutions
Proteins not transferred during Western blotting	• Staining proteins to check transfer efficiency • Optimize the acrylamide concentration in the gel, the transfer time, the voltage using markers covering the expected molecular weight (the molecular weight and the Stoke radius of the protein affect the transfer). You can also use a known protein as a positive control

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Too few antigens or antibodies	• Increase the amount of antigen-carrying proteins of interest • Increase the concentration and incubation time of the primary antibody. Optimization may be required
Weak binding of the primary antibody	• Ensure that the antigenicity of the protein has not been affected by the treatments inherent in electrophoresis (SDS, urea, denaturation, etc.). Verify the ability of the antibody to bind to the antigen by Dot blotting
HRP in excess	• An excessive amount of HRP can cause the **Owi ECL RevelBIOt® Femto substrate to deplete** and the signal to be rapidly switched off. Dilute the HRP-conjugated antibody at least 10-fold
Decreased HRP or substrate activity **Owi ECL RevelBIOt® Femto**	• Verify that the **Owi ECL RevelBIOt® Femto** active solution and solutions (Luminol/Enhancer) and B (Peroxide buffer) have been stored and used in good conditions • Test HRP activity: Prepare a small volume (1 ml) of **Owi ECL RevelBIOt® Femto** Active Solution. In the dark, add 1 µL of undiluted HRP conjugated antibody. Blue light should be visible to the naked eye

9.3 Non-specific bands

Cause possible	Notes / Solutions
HRP in excess	• Dilute the HRP-conjugated antibody at least 10-fold

9.4 Uneven signal distribution

Possible causes	Notes / solutions
Inefficient protein transfer	• See above (no signal)
Membrane not homogeneously immersed	• Start over with a new membrane • Make sure the membrane is completely covered at all times during incubation
Contamination with fingerprints and/or keratin	• Avoid touching the membrane. Use gloves and tongs
Presence of bubbles between the film and the membrane	• Remove bubbles. Exhibit again

9.5 White (negative) stripes on the film

Cause possible	Notes / Solutions
HRP in excess	• Dilute the HRP-conjugated antibody at least 10-fold

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