Owi ECL RevelBIOt® Pico

Chemiluminescent substrate
User Manual

Reference: OZYB001-2500

For 2500 cm2 membrane (2x 125 ml)
Storage and stability: **one year at room temperature**

FOR RESEARCH ONLY

DOMINIQUE DUTSCHER SAS

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SECTION 1: DESCRIPTION

Owi ECL RevelBIOt® Pico is a chemiluminescent substrate for HRP. It enables the detection of membrane-immobilized antigens (Western blotting) with a sensitivity threshold in the picogram range.

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 blocking agents and empirically determine the most suitable one.
  - Add Tween-20® to antibody blocking and dilution buffers to reduce non-specific signals.

Throughout the protocol, the membrane must never dry out. Ensure that you use the appropriate amount of buffers, including ~~the~~ blocking buffer, washing buffer, diluted antibody solution, and the **Owi ECL RevelBIOt® Pico** working solution.

- 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 preservative in buffers, as it is an HRP inhibitor
- Use clean utensils. Rust stains on scissors or forceps can cause a high background noise.
- The **Owi ECL RevelBIOt® Pico** working solution is stable for 8 hours at room temperature. If not used immediately, store it in an opaque bottle

SECTION 3: COMPONENTS

	Solution A - Brown Bottle	Solution B - Clear Bottle
OZYB001-2500 For 2500 cm2 of membrane	125 ml Luminol/Enhancer	125 ml Peroxide Buffer

SECTION 4: WORKING SOLUTIONS

- Dilution Buffer: Tris-Buffered Saline (TBS)
- **Washing 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 at 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 1:500 and 1:5000 or 0.2-2 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 1:20,000 and 1:100,000 or 10 to 50 ng/ml.

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- **Owi ECL RevelBIOt® Pico** working solution: It is obtained by mixing equal volumes (50/50) of Solution A (Luminol/Enhancer, contained in the brown bottle) and Solution B (Peroxide buffer, contained in the clear bottle).

SECTION 5: MATERIAL REQUIRED

- Protein electrophoresis tanks, Western blot membrane transfer device.
- Nitrocellulose membrane (or PVDF) for Western blot application.
- Stirring platform or equivalent system.
- Detection:
  - Imager with a CCD camera compatible with low-signal detection and configured for Western blot,

  or
  - X-ray sensitive film, cassette & dedicated fixing and developing reagents for 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	2 minutes incubation with **Owi ECL RevelBIOt® Pico** working 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 blotting

SECTION 7: COMPLETE WESTERN BLOT PROTOCOL

Step 1 – Separate the protein of interest by electrophoresis.

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

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

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

Step 5 – Wash the membrane with the washing buffer on a stirring platform or an equivalent system, performing at least 4 to 6 washes, each lasting 5 minutes or more at room temperature with fresh washing buffer. Increasing the volume of washing buffer and the number of washes helps to reduce background noise.

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

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

Step 8 – Incubate the membrane in the **Owi ECL RevelBIOt® Pico** working solution (see section "Working Solutions") for 2 minutes. The total volume must cover the membrane; Under standard conditions, use 0.1 ml of **Owi ECL RevelBIOt® Pico** working solution per cm² of membrane. Owi ECL RevelBIOt® Pico working solution is stable for 8 hours at room temperature. Incubation can be done in daylight or in a normally lit room, but should be away from intense light sources. If not used immediately, protect **the Owi ECL RevelBIOt® Pico** working solution from light by stirring it in an opaque bottle.

Step 9 – Remove the membrane from the **Owi ECL RevelBIOt® Pico working 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 X-ray film :
    - Place the wrapped 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 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 lasts for several hours with a peak intensity 5 to 30 minutes after incubation with the **Owi ECL RevelBIOt® Pico,** then gradually decreases over time. If needed, additional exposures can be performed using new films. Due to the half-life of the HRP, longer exposure times may be required.
    - Develop the film using the appropriate fixing and developing reagents

- Imager Reveal: Use an imager equipped with a CCD camera configured for Western blot detection.
    - Exposure time varies by device and may be longer than with autoradiographic film.

Note: at the end of the protocol, you can store the membrane wrapped in a transparent film type "Saran Wrap" at +2°C 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, pH 6.7) for 30 minutes at 50°C. For more stringent conditions, incubation can be performed at 70°C for more than 30 min.

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

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

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

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SECTION 9: TROUBLESHOOTING

9.1 High Background

Possible causes	Notes / Solutions
Antibody concentrations too high	• Dilute primary and/or secondary antibody concentrations
Inefficent blocking agent	• Check 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: 1-10% (je laisserai 1 to 10%) BSA to TBS-Tween or PBS-Tween (freshly prepared); 0.5% to 3% gelatin in TBS-T or PBS-Tween (freshly prepared)
Inefficient washing	• Increase washing time and washing buffer volume • Add Tween (if not already used) • Increase the concentration of Tween in the washing buffer
Contaminated Buffers	• Ensure all buffers are freshly prepared and filtered
Contamination of equipment (tanks/transfer device)	• Clean or replace devices
Membrane issues	• Ensure the membrane is completely submerged and remains well moistened in all solutions • Use high-quality nitrocellulose (or PVDF) membranes • A damaged membrane can cause non-specific antibody binding. • Handle the membrane with care using powder-free gloves (see "Precautions" section) • Use clean forceps to handle the membrane after washing
Owi ECL RevelBIOt® Pico Detection Solution	• Excess **Owi ECL RevelBIOt® Pico** working solution. Remove excess with absorbent paper 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 it 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	• Stain the 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 Stoke's radius of the protein affect the transfer). You can also use a known protein as a positive control.
Insufficient quantity of 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.). Check the binding ability of the antibody to the antigen by Dot Blot.
Excess HRP	• Excessive HRP can cause depletion of the **Owi ECL RevelBIOt® Pico** substrate and rapid signal extinction. Dilute the HRP-conjugated antibody at least 10-fold.

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Decreased activity of HRP or **Owi ECL RevelBIOt® Pico** substrate	• Check that the **Owi ECL RevelBIOt® Pico** working solution and solutions A (Luminol/Enhancer) and B (Peroxide buffer) have been stored and used in appropriate conditions. • Test HRP activity: Prepare a small volume (1 ml) of **Owi ECL RevelBIOt® Pico 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

Possible causes	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 evenly immersed	• Restart with a new membrane • Make sure the membrane is fully covered at all times during incubation
Contamination from fingerprints and/or keratin	• Avoid touching the membrane. Use gloves and forceps
Presence of bubbles between the film and the membrane	• Remove bubbles. Exhibit again

9.5 White (negative) stripes on the film

Possible Cause	Notes / Solutions
Excess HRP	• Dilute the HRP-conjugated antibody at least 10-fold

SECTION 10: RELATED PRODUCTS

ECL	
OZYB002-1000	Owi ECL RevelBlOt Femto 100 ml (Mid to low femtogram detection)

PROTEIN MARKER	
OZYC005-500	Owi ECOlor Prestained Protein Ladder

Version: OZYB001-473885

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