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ECL One-Star

One Solution Chemiluminescent Substrate

Instruction For Use

RUO

ECL ONE- STAR CHEMILUMINESCENT SUBSTRATE SOLUTION

Reference	Size
EMP003010	Sufficient for 100 cm^2 of membrane (SAMPLE)
EMP003250	Sufficient for 2500 cm^2 of membrane

FOR RESEARCH USE ONLY

This product is intended for research use only. Not for use in diagnostic procedures.

1. MATERIALS SUPPLIED

Reference	One Solution: Luminol/Enhancer/ Peroxide Buffer
EMP003010	10 ml
EMP003250	250 ml

2. STORAGE AND STABILITY

One year at room temperature 18-25°C.

3. INTRODUCTION

ECL One-Star, a “Ready to Use” Solution Chemiluminescent Substrate, is a non-radioactive light emitting substrate for the detection of picograms amounts of immobilized specific antigens, conjugated directly or indirectly with horseradish peroxidase HRP-labelled antibodies. The premixed solution allows an increased experimental consistency avoiding pipetting errors and possible contaminations.

4. IMPORTANT NOTES

• Optimal antibodies dilutions may vary among different applications depending on quality and affinity for the target protein. It is crucial to optimize both primary and secondary Ab dilutions for best results with high signal and low background.
• To achieve the highest signal-to-noise ratio it is strongly recommended to test different blocking reagents and empirically determine which is the most suitable for the Western Blot systems you are using.
• Addition of Tween®-20 to blocking and antibody dilution buffer reduces non specific signal.
• Throughout the procedure the blot membrane should never dry out: use always a proper amount of either blocking buffer, washing buffer, antibody solution and ECL One-Star Substrate Solution. For optimal results perform incubation steps on a shaking platform.
• When handling film or detection reagent always wear powder-free gloves or use forceps.
• Do not use azide as a preservative for buffers as it is an inhibitor of horseradish peroxidase.
• Any equipment used for membrane treatment must be clean and free of foreign material. Traces of rust on either scissors, forceps or other metallic devices may cause high background.

5. MATERIALS REQUIRED BUT NOT SUPPLIED

Equipment:
• Electrophoresis and electrotransfer apparatus for Western Blot
• Nitrocellulose membrane specifically intended for Western Blot
• Shaking platform
• Film cassette, x-ray film, developing and fixing reagents, or imaging system

Working solutions:
• Dilution Buffer: Tris-buffered Saline (TBS)
• Washing Buffer: TBS-Tween 0.1%
• Blocking Buffer: Dissolve the proper blocking agent (Non Fat Dried Milk, BSA, etc.) in TBS-Tween 0.1%.
• Primary antibody: Dilute the antibody specific for the target protein in dilution buffer in order to get a 1 mg/ml stock solution. Use Blocking Buffer to prepare the antibody working dilution. The suggested working dilution range is between 1:100 and 1:5.000.
• HRP-secondary antibody: If the HRP-conjugated antibody is supplied as 1 mg/ml stock solution, the suggested working dilution range is between 1:1.000 and 1:15.000. Use Blocking Buffer to prepare the antibody working dilution.

6. PROTOCOL AT A GLANCE

Step 1: Protein separation by electrophoresis
Step 2: Protein transfer to membrane
Step 3: Blocking of non-specific sites
Step 4: Membrane incubation with the primary antibody specific for the antigen to be detected

Step 5: Membrane wash
Step 6: Membrane incubation with HRP-labeled secondary antibody
Step 7: Membrane wash
Step 8: Membrane incubation with ECL One-Star substrate for 1,5 minutes
Step 9: Membrane exposure to X-ray film or imaging instrument

7. PROTOCOL

7.1 - Perform electrophoresis.
7.2 - Transfer proteins to nitrocellulose or PVDF membrane.
7.3 - Incubate the membrane in blocking buffer for 1 hour on a shaking platform at room temperature. Membranes may be incubated in the blocking solution over night at 2-8°C if this is more convenient.
7.4 - Remove blocking reagent. Incubate the membrane in diluted primary antibody for 1 hour on a shaking platform. Please use the dilution factor suggested in the Working Solutions Section.
7.5 - Wash membrane with washing buffer. Perform at least 4-6 washes of ≥ 5 minutes on a shaking platform. Each wash must be done with fresh washing buffer at RT. To reduce background increase the number of washes and the buffer volume.
7.6 - Rinse membrane and incubate in diluted HRP-labeled secondary antibody for 1 hour on a shaking platform. Please use the dilution factor suggested in the Working Solutions Section.
7.7 - Wash the membrane (repeat step 7.5)
7.8 - Use a volume of 0.1 ml/cm^2 of membrane of ECL One-Star Solution, ensuring that the whole membrane is covered. Incubate the membrane in for 1,5 minutes. Intense light can harm the Solution, while typical laboratory light will not alter it.
7.9 - Remove the membrane from ECL One-Star Solution. Drain off excess detection reagent by holding the membrane vertically and touching edge on tissue paper. Place the membrane in a plastic membrane protector (plastic wrap may also be used) paying attention to remove excess of liquid and air bubbles. Place the wrapped membrane in the film cassette with the protein side facing up. Ensure that there is no free detection reagent in the film cassette: the film must remain dry during exposure. Switch off lights and place a sheet of film on top of the membrane, close the cassette and expose. Do not move the film during exposure to avoid any artifacts on the film. Expose for 60 seconds. Exposure time may be varied to achieve optimal results. If using a phosphor imaging system or a CCD camera longer exposure times may be necessary. Remove film and immediately place with a fresh piece of unexposed film and reclose film cassette. Light emission will last up to 3 hrs but the highest intensity is during the first 60 minutes after substrate incubation. Longer exposure times may be necessary as the blot ages. Develop film using appropriate developing solutions and fixative.

After ECL One-Star detection, is possible to store the wrapped membrane at 2-8°C. The membrane can then be stripped and re-probed several times (use StripAblot EMP100500).

8. TROUBLESHOOTING

High Background	
Possible Cause	**Precautions/remedies**
Antibody concentrations are too high	Very high concentrations of both primary and/or secondary antibodies can cause high background. Decrease antibody dilutions.
Inadequate blocking	Check that blocking agent solution has been made up correctly.
	Include blocking agent in all detection reagent working solutions.
	Try alternative blocking agents: 1-10% bovine serum albumin in TBS-T or PBS-T (freshly prepared) 0.5-3% gelatin in TBS-T or PBS-T (freshly prepared).
Primary antibody is not specific for the protein of interest	Use monospecific or antigen affinity purified antibodies. Always incubate your primary antibody at 4°C overnight and not at room temperature. Reduce NaCl in TBS-T Buffer (100 mM÷350 mM). Use monospecific or antigen affinity purified Ab.
Non-specific binding of secondary antibody	Confirm the secondary is specific by omitting the primary and running a secondary only blot. If bands develop choose an alternative secondary antibody.
Inadequate washing	Increase washing times and volumes of wash buffer.
	Add Tween®20 to reagents if not yet included.
	Increase concentration of Tween®20 in washing solution.
Contaminated buffers	Ensure all buffers are freshly prepared and filtered.
Contaminated blot equipment	Clean or replace all equipment.

Problems with membranes	Check that the membranes are completely soaked in all solutions especially during washing, and that membranes hydrate thoroughly.
	Use high quality nitrocellulose membranes.
	Damage to the membrane can cause non-specific binding of the detection reagents. Handle blots carefully with gloved hands.
	Use clean forceps to handle blots after washing.
Detection reagents	Excess detection reagents in blots. Drain well by absorbing the excess on tissue paper before placing blots in film cassettes.
Over exposure	Expose the film for a minimum period (an initial 15 seconds exposure may be all that is required). If exposure time is too short to be convenient, reduce antibody concentrations.
	Leave blots in the cassette for 5-10 minutes before re-exposing to film.
Weak or No signal	
Possible Cause	**Precautions/remedies**
No transfer of proteins during Western Blot	Stain membrane with protein stain to check for transfer efficiency.
	Optimize gel acrylamide concentration, time for transfer and currently use molecular weight markers covering the molecular weight range expected to be blotted.
Insufficient quantities of antigen or antibody	Increase amount of antigen.
	Increase and optimize concentration and incubation times of primary antibody.
Poor binding of primary antibody	Check that antigenicity is not destroyed by treatments required for electrophoresis (SDS, UREA, boiling etc). Check the antigen binding capacity of the antibody using a dot blot system.
Sodium azide contamination	Make sure that buffers do not contain sodium azide as this will quench HRP signal.
Contaminated stock solutions	Do not contaminate the chemiluminescent substrate stock solution using the same pipette tip. Use new reagents.
Too much HRP in the system	Excess of HRP can deplete the substrate and cause the signal to fade quickly. Dilute HRP-conjugate at least 10-fold.
Reduction of HRP or substrate activity	Check that the detection reagent is stored correctly and used as recommended.
	Test the activity of the system: in the dark, add 1 µl of undiluted HRP-labeled antibody to a small volume (1 ml) of ECL One-Star solution. Visible blue light should be produced.
Irregular black spots	
Possible Cause	**Precautions/remedies**
Air bubbles trapped in membrane	Remove air bubbles by gently rolling a clean serological pipette or a test-tube during sandwich assembling.
Unevenly hydrated membrane	Make sure that the membrane is fully immersed during washes and antibody incubations.
Contaminated equipment	Protein or pieces of gel remaining on the unit may stick to the membrane. Antibody can get trapped in the gel, and then are washed out poorly, resulting in intense localized signal.
Aggregation of blocking agent	When blocking agent is in powder form, stir it over night at 4°C to make sure it is completely dissolved.
Interaction of the membrane with sample tray	Always use clean plastic trays to avoid any type of cross-reaction.
Formation of aggregates in HRP-conjugate	Filter secondary antibody solution through a 0.2 µm filter. Use fresh antibody.
White bands or “ghost bands”	
Possible Cause	**Precautions/remedies**
Excessive signal generated	Excessive antibodies or loaded protein can cause high levels of localized signal. This results in rapid consumption of substrate at this point. Since there is no light production after the completion of this reaction, white bands are the result. Try first to further dilute secondary antibody.

Uneven or jagged bands	
Possible Cause	**Precautions/remedies**
Uneven gel run	Load all available wells. Empty wells can be loaded with sample buffer.
Voltage or current were too high during electrophoresis	Reduce voltage or current during electrophoresis.
Effects of high salt in samples	Reduce NaCl concentration in TBS-T Buffer (100 mM÷350 mM).

For further product information, contact Euroclone Technical Assistance Department:
E-mail: tsa@euroclone.it
T: +39 02381951
Website: www.euroclone.it

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