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Datasheet

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ACEsignal Chemiluminescent Substrate - Femto

Cat# A1042– 500 ml

Storage at 4°C

INTRODUCTION

ACEsignal Chemiluminescent Substrate - Femto is an extremly sensitive ECL product by supplementing a proprietary luminol for chemiluminescent detection of immobilized proteins (Western blotting), conjugated with Horseradish Peroxidase (HRP) directly or indirectly. In Western blotting application, **ACEsignal Chemiluminescent Substrate - Femto** provides high signal and low background, which allows detection of protein targets at femtogram levels. This feature benefits the researchers with excellent low background results and without signal burn effects at the same time.

CONTENTS

No	Component	A1042– 500 ml
AA	Solution A (Luminol Solution)	250 ml
AB	Solution B (Peroxide Solution)	250 ml

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

ACEsignal Chemiluminescent Substrate - Femto 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 **Blocking Buffer for WB, ELISA and IHC (A1009)** 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 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 °C for 8-12 hours.
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, secondary antibody dilution can start from 1:40,000.

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 cm^2 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 1 minute as the initial exposure time.

D. Striping 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 ACEsignal Chemiluminescent Substrate - Femto 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 work 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	Shorten the exposure time

RELATED PRODUCTS

ACEsignal Chemiluminescent Substrate - EcoPlus	A1040	500 ml
ACEsignal Chemiluminescent Substrate - Pico	A1041	500 ml
1 Min Stripping Buffer	A1039	500 ml
Protein-Free blocking buffer	A1010	1 L
Blocking Buffer for WB, ELISA and IHC	A1009	1 L
BCA Protein Assay Kit	A1035	500 ml
Bradford protein assay (5X)	A1034	500 ml