LumiPico® ECL Kit

For Western Blot

User Manual

Cat.Nos.ZK00901(12.5ml×2) **USD44.46**

ZK00902(50.0ml×2) **USD175.20**

Published 24 Feb 2007

Description:

LumiPico® is a complete kit with ready-to-use reagents for chemiluminescent detection of immobilized proteins (Western blotting) or immobilized nucleic acids (Southern or Northern), conjugated with HRP directly or indirectly.

Theory:

In the presence of hydrogen peroxide (H2O2), Horseradish peroxidase (HRP) catalyzes the oxidation of luminol. Immediately following the oxidation, the luminol decays and emitting light, and the light length is 428nm. Using this method, it is possible to detect membrane immobilized specific antigens, or sequences of nucleic acids, labeled directly with HRP or indirectly with HRP-labeled antibodies.

Advantages:

- High sensitivity non-radioactive detection system.
- Stable hard copy results on film.
- Only small amounts of antibody required.
- Detection may be achieved in short exposure times (minutes)
- High resolution.

Caution:

If above solutions come into contact with eyes or skin, flush with plenty of water and remove contaminated clothing.

Protocol for Western Blotting and Chemiluminescence Detection

1.Preparation of Solutions

See general buffer solution at the end of this file.

2. Electrophoresis, Blotting and Membrane Preparation

2.1Carry out electrophoresis for protein separation.Either non-denaturing gel, SDS-PAGE or two dimensional gels may be used.

2.2.Transfer proteins from the gel to a membrane. Use nitrocellulose or PVDF membrane. PVDF membranes must be wetted briefly in methanol then soaked in distilled water for 1-3 minutes, followed by equilibration in transfer buffer.

2.3 Block non-specific binding sites by incubating the membrane for 1 hour at room temperature with shaking in TBST solution containing 5% dried milk (w/v). This step can be performed overnight at 4°C without shaking.

2.4 Dilute the primary antibody in TBST with 2% dried milk (w/v).Incubate the membrane in the solution for 1 hour at room temperature with shaking, or overnight at 4°C without shaking.

2.5 Wash the membrane three times in TBST for 10 minutes each. Use at least 50ml of buffer for 10x10cm membrane.

2.6 Dilute the HRP-labeled secondary antibody in TBS-T with 2% dried milk (w/v). Incubate the membrane in the solution for 1 hour at room temperature with shaking.

2.7 Wash the membrane as detailed in 2.5.

3. Enhanced Chemiluminescence Detection

3.1 Preparations

Prepare the following equipment and solutions in a dark room:

- X-ray film cassette
- X-ray film
- Timer
- Developer, fixer and water in tanks
- Transparent plastic bag or saran wrap
- Glass pipettes
- Sterile gloves - to prevent hand contact with membrane, film or reagents

3.2 Detection

3.2.1 Mix an equal volume of LumiPico® Solution A and Solution B to give sufficient solution to cover the membrane (0.1ml/cm^2). Let the detection mix equilibrate for at least 5 minutes.

3.2.2 Drain the excess buffer from the washed blots. Do not let the membrane dry out. Add the detection mix directly to the blot (protein side up). Incubate for 1-3 minutes at room temperature.

3.2.3 Drain off excess detection mix and wrap the membrane in saran wrap. Gently remove air pockets.

3.2.4 Place the blots, protein side up, in the film cassette. Switch off the lights and use red safety light. Place a sheet of film on the blot, close the cassette and expose for 30-60 seconds.

3.2.5 Replace the exposed film with a new one, close the cassette and develop the first exposed film.

3.2.6 Expose the second film for a suitable time according to the signal intensity on the first film.

3.2.7 If signal intensity was too high, wait up to 30 minutes before re-exposing.

4. Optimization of Antibody Concentration for LumiPico®. It is essential to optimize the immunoblot conditions to achieve maximum signal and minimum background. First optimize the concentration of the primary antibody using a constant amount of secondary-HRP conjugate. Using the optimized primary antibody concentration, adjust the concentration of the secondary antibody-HRP conjugate.

4.1 Dot-Blot for Primary Antibody Optimization

Prepare one piece of nitrocellulose membrane for each primary antibody dilution to be tested.4.1.1 Spot a dilution range of protein onto the membrane.

4.1.2 Allow the membrane to air-dry.

4.1.3 Block non-specific binding sites by incubating the strip for 1 hour at room temperature with shaking in TBST solution containing 5% dried milk (w/v). This step can be performed overnight at 4°C without shaking.

4.1.4 Prepare several dilutions of primary antibody in TBST with 2% dried milk (w/v), (e.g. 1:100-1:5,000). Incubate one piece of membrane in each dilution for 1 hour at room temperature with constant shaking, or overnight at 4°C without shaking.

4.1.5 Wash the membranes three times in TBST for 10 minutes each. Use at least 0.5ml of buffer per 1cm membrane.

4.1.6 Dilute the HRP-labeled secondary antibody in TBST with 2% dried milk (w/v) to the known optimal dilution. Incubate each strip in the solution for 1 hour at room temperature with shaking.

4.1.7 Wash the membranes as detailed in 4.1.5 above.

4.1.8 Detection: as detailed in 3.2 above.

4.2 Dot-Blot for Secondary Antibody Optimization

Prepare one piece of nitrocellulose membrane for each secondary antibody dilution to

be tested.

4.2.1 Prepare dot-blots as detailed in 4.1.1 - 4.1.3 above.

4.2.2 Dilute the primary antibody in TBS-T with 2% dried milk (w/v) to the known optimal dilution. Incubate each strip in the solution for 1 hour at room temperature with shaking.

4.2.3 Wash the membranes as detailed in 4.1.5 above.

4.2.4 Prepare several dilutions of secondary antibody in TBS-T with 2% dried milk (w/v), (e.g. 1:5,000-1:100,000). Incubate one piece of membrane in each dilution for 1 hour at room temperature with constant shaking.

4.2.5 Wash the membranes as detailed in 4.1.5 above.

4.2.6 Detection: as detailed in 3.2 above.

5. Stripping and Reprobing of Membrane

The immunoblot can be stripped of blocking reagent and antibodies, and then reprobed as required.

5.1 Incubate membrane in stripping buffer for 30 minutes at 50-70°C.(62.5mM Tris-HCl pH 6.8, 100mM β-mercaptoethanol and 2% (w/v) SDS).

5.2 Wash the membrane twice in TBS-T for 10 minutes each. Use at least 50ml of buffer for 10x10cm membrane.To ensure removal of antibodies, incubate the membrane with LumiPico® detection reagents and expose against film. Repeat previous steps if a signal is detected.

5.3 Reprobe the blot as detailed in 2.3 - 3.2.7 above.

Common buffers:

Buffers for Western blot

RIPA

Tris.HCl， 50 mmol/L， pH 7.5;

NaCl， 150 mmol/L;

NP-40，1%;

C24H40O4·Na，5%;

SDS， 0.1%;

EDTA， 1 mmol/L;

PMSF， 1 mmol/L;

Leupeptin， 2 μg/ml

1xSample buffer:

50mM	Tris-Cl pH 6.8
2%	SDS
10%	glycerol
0.02%	Bromophenol blue
0.7%	2-mercaptoethanol

Running buffer:

Tris-base 3.g
glycine 14.4g
10%SDS 10ml
add ddH_2O to 1000ml, Do not adjust the pH!!

Transfer buffer:

Tris-base 3.g
Glycine 14.4g
methanol 200ml
add ddH_2O to 1000ml, Do not adjust the pH!!

Blocking Solution:

For 2 blots, prepare 100 ml:
Weigh 5 g skim milk powder into a beaker.
Add 100 ml TBS-T and stir to suspend completely.

TBST buffer:

20mM Tris-Cl pH 7.4
0.15M NaCl
0.5%Tween-20 1~5ml

add ddH_2O to 1000ml, Do not adjust the pH!!

Ponceau Stain:

Dissolve 0.5 g Ponceau S in 1.0 ml glacial acetic acid. Bring volume to 100 ml with MQ water. Final solution is 0.5% Ponceau/1% acetic acid. Wrap bottle in foil to protect from light. Reuse the solution until staining is no longer as good.

Stripping buffer:

1) In graduated cylinder, add 50ml of 10x Tris Buffer, pH 6.8 (500mM), to 300ml ddH2O.
2) Add 100ml 10% SDS
3) Add 3.5ml BME
4) Bring up to 500ml with ddH2O.

STRIP DEVELOPED BLOTS:

1) Place blot in a 50ml conical tube filled with strip buffer.
2) Seal with parafilm and put in water bath set at 50°C for 30 minutes. Shake gently every 10 min.
3) Decant Strip Buffer. Rinse with TBST approximately 5 minutes.
4) Repeat 3 times (4 rinses total).
5) Store in TBS at 4°C or proceed to standard immunoblotting protocol.

Reference:

Blake, M.S., et al. A Rapid, *Sensitive Method for Detection of Alkaline Phosphatase Conjugated Antibody on Western Blots.* **Anal. Biochem.**, 136:175-178, 1984.

Bittner, M., P. Kupferer, and C. F. Morris. 1980. *Electrophoretic Transfer of Proteins and Nucleic Acids from Slab Gels to Diazobenzyloxymethyl Cellulose or Nitrocellulose Sheets.* **Anal. Biochem.**, 102:459-471.

Burnette, W.N. 1980. *Western Blotting: Electrophoretic Transfer of Proteins from Sodium Dodecyl Sulfate-Polyacrylamide Gels to Unmodified Nitrocellulose or Nitrocellulose Sheets.* **Anal. Biochem.**, 112:195-203.

Shanghai ShineGene Molecular Bio-tech Co.,Ltd.

Add: Floor 2, Building A, 328#, Wuhe Road, Shanghai, 201109, China

Tel: +86-21-54460831 800-988-1995

Fax: +86-21-54460832

E-mail: master@shinegene.org.cn

Website：www.shinegene.org.cn