ab133408 –

Optiblot ECL Max Detect Kit (4.6pg – 4.7ng)

Instructions for Use

For enhanced chemiluminescent
Western blotting.

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Western blotting is a protein analysis tool that has become commonplace in molecular biology and protein chemistry laboratory. The principle of chemiluminescent Western blotting is shown in Figure 1. Proteins are separated by size via electrophoresis, and then transferred electrophoretically from the gel to a membrane support, usually nitrocellulose or PVDF. This membrane containing the transferred proteins is commonly referred to as a blot. The location of a protein of interest is detected on the blot by applying the primary antibody, which binds to the protein. The primary antibody bound to the blot is then visualized using a secondary antibody that binds to the primary antibody. The secondary antibody is labeled in some way to make it detectable, such as with a radioactive isotope or an enzyme that can be detected by its activity.

Since 1988, enhanced chemiluminescence or ECL has become one of the most common detection methods in Western blotting. In this method, the secondary antibody is conjugated to the enzyme Horseradish peroxidase. Once bound to the membrane, the secondary antibody is detected by incubating the blot with a solution containing an HRP substrate that generates a light-emitting product after reaction with HRP (Figures 1, 2). The chemiluminescent signal can be detected by exposing the blot to X-ray film or by imaging with a CCD camera.

Optiblot ECL Max Detect Kit (ab133406) is a new horseradish peroxidase (HRP) substrate specially developed to tak e advantage of the large linear dynamic range provided by CCD imaging.

Optiblot ECL Max Detect Kit produces a strong, extreme ly long-lived signal, which, combined with very low background levels, allows for long exposure times enabling the detection of low -abundance proteins. Additionally, the signal from Optiblot ECL Max Detect Kit is linear with respect to protein amount over a broad range of concentrations, displaying no substrate depletion at high protein loads, allowing the user to take full advantage of the linear range of the CCD detection method.

Optiblot ECL Max Detect Kit is also compatible wit h X-ray film detection, though the limited dynamic range of film will make resulting data less quantitative.

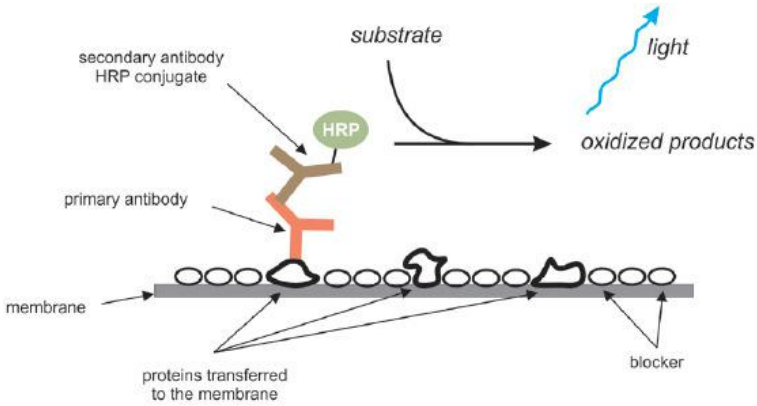

Figure 1. The principle of chemiluminescent Western Blotti ng.

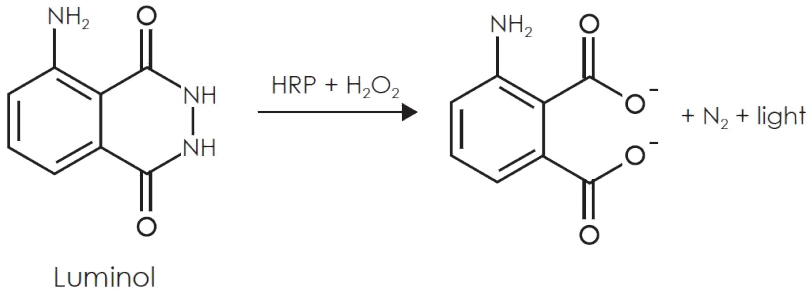

Figure 2. Chemiluminescence of luminol

2. Assay Summary

Electrophoresis

to separate proteins in sample

Transfer

proteins from gel to membrane

Block

to mask non-specific protein binding sites on membrane

Primary Antibody

binds to protein of interest

Wash

to remove excess antibody

Secondary Antibody

binds to primary antibody

Wash

to remove excess antibody

Substrate (Optiblot ECL Max)

substrate reacts with HRP bound to secondary antibody to create luminescent signal

Image

detect luminescent signal with CCD camera or film

3. Kit Contents

The Optiblot ECL Max Detect Kit contains sufficient substrate for 200, 1000 or 2000 cm^2 of membrane surface. The following components are included:

Item	Size (20 mL)	Size (100 mL)	Size (200 mL)
Luminol/enhancer solution	10 mL	50 mL	100 mL
Peroxide Chemiluminescent Detection Reagent	10 mL	50 mL	100 mL

4. Storage and Handling

The Optiblot ECL Max Detect Kit reagents are stable at room temperature for one year. Shelf life can be extended if the product is stored refrigerated at 4°C. Accidental freezing does not significantly affect the performance, but, multiple freezing-thawing cycles are not recommended.

5. Additional Materials Required

- Electrophoresis apparatus and buffers for SDS-PAGE.
- Tank and buffers for electrophoretic transfer of proteins from gel to membrane.
- Nitrocellulose or PVDF membrane, cut to size of gel.
- Washing buffer (PBS-T or TBS-T).
- Blocking buffer
- Primary antibody compatible with your application.
- Secondary antibody, conjugated to Horseradish peroxidase (HRP) corresponding to your primary antibody.
- CCD-based detection system, or film

6. Protocol

1. Prepare a protein blot

1.1. Separate protein sample(s) via electrophoresis

1.2. Transfer proteins to membrane

- Pre-wet membrane in transfer buffer and assemble transfer sandwich according to manufacturer's instructions.
- Dot-blots or slot blots can also be detected with Optiblot ECL Max Detect Kit.

Notes:

Any electrophoresis system and buffer is compatible with Optiblot ECL Max Detect Kit.

A wet or tank transfer method is preferred, though semi-dry methods should also be compatible.

Both nitrocellulose and PVDF membranes are compatible with Optiblot ECL Max Detect Kit.

If using PVDF, first wet membrane with a 1 min incubation in 100% MeOH followed by water for ~5 min and then transfer buffer for 5-10 min.

For slot blot applications, nitrocellulose is much more convenient than PVDF because it is more difficult to avoid bubbles with PVDF.

2. Block Membrane

2.1. Incubate the blot in a blocking buffer with gentle agitation for 1 hour at room temperature (RT). Use 0.2 to 0.5 ml of blocking buffer per cm^2 of blot to provide adequate blocking.

Notes:

Blocking masks non-specific protein binding sites on the membrane, reducing background and increasing the specificity of binding of the primary antibody to the protein of interest.

The optimal blocking buffer will depend in part on the nature of the antigen of interest, and on the quality of the primary antibody. Common blocking agents including non-fat dry milk have been found to be compatible with Optiblot ECL Max Detect Kit.

10 to 20 ml is usually sufficient for a typical 7 x 9 cm mini-blot.

3. Incubate blot with primary antibody

3.1. Dilute primary antibody in blocking buffer

3.2. Incubate blot with primary antibody solution for 1 hour at RT with gentle agitation.

Notes:

Optimal primary antibody dilutions must be determined empirically.

For CCD imaging, we recommend primary antibody dilutions from 1:1000 to 1:10,000. A good initial dilution is 1:5000

If the blot will be imaged on film, use 2-5x less primary antibody than for CCD imaging. For example, if 1:1000 dilution of the primary antibody was optimal for CCD detection, 1:5000 is suitable for film detection.

Antibody can be added to a dish and placed on a shaker, or a smaller volume (5-10 ml) can be used by sealing the blot into a bag and placing it on a rotary platform.

4. Wash blot to remove excess primary antibody

4.1. 1 x quickly

4.2. 1 x 15 min, with 0.7 ml/cm membrane

4.3. 3 x 5 min, with at least 0.3 ml/cm^2 membrane each time.

Notes:

PBS-T or TBS-T are compatible with Optiblot ECL Max Detect Kit.

We recommend washing or rocking blots in a clean dish on a shaker to provide gentle agitation.

For example, a standard 7x9 membrane requires

~50 ml of washing solution for the 15 min wash and

~20 ml of washing solution for 5 min washes

5. Incubate blot with secondary antibody

5.1. Dilute secondary antibody in blocking buffer.

5.2. Incubate blot with secondary antibody solution for 1 hour at RT with gentle agitation.

Notes:

Optimal secondary antibody dilutions must be determined empirically.

We recommend secondary antibody dilutions of 1:5,000 to 1:20,000. A good initial dilution is 1:10,000.

If the blot will be imaged on film, use 2-5x less secondary antibody than for CCD imaging. 1:50,000 dilution is a good starting point for film detection.

See also notes for step 3.

6. Wash blot to remove excess secondary antibody

6.1. 3 x 5 min, with at least 0.3 ml/cm^2 membrane each time

See notes for step 4.

7. Incubate blot with Optiblot ECL

7.1. Mix components 1 and 2 in a 1:1 ratio in sufficient amounts to obtain at least 0.1 ml/cm^2 of the blot and add to the blot.

7.2. It is better to prepare the working solution just before use. However, mixed reagent is stable for several hours at RT.

7.3. Allow substrate to react with blot for 2 minutes.

Notes:

Be careful not to touch or put pressure on the blot as this can result in non-specific background.

Use only plastic forceps, not metal; metal forceps damage the blocked surface, creating new adsorption sites. Also, traces of metal may act as a catalyst for non-enzymatic substrate oxidation, resulting in very high background.

The minimal amount of working reagent is 0.1 ml/cm^2. For example, for a 7 x 9 cm blot, this minimal volume is 7 x 9 x 0.1 = 6.3 ml.

If using the minimal amount of working reagent, incubation may be done without agitation. Make sure the membrane surface is level so adequate reagent is held by surface tension.

Incubation may also be done with gentle agitation in a tray just slightly larger than the membrane. Increase the reagent volume as necessary to ensure the membrane is adequately covered with reagent.

8. Drain excess reagent

8.1. Remove excess substrate via capillary action by touching absorbent material to the edge of the blot.

9. Image blot

9.1. While blot is damp, cover with transparent plastic wrap and either place blot in CCD imager, or expose blot to film.

Notes:

We recommend trying three exposures; 30 sec, 2 min, and 5 min.

The blot can be imaged and re-imaged for several hours; 70% of the initial signal will remain after 60 minutes, and substantial signal will remain after 8-10 hours.

7. Troubleshooting

Western blotting can require substantial optimization due to the multiple steps involved. The correct amount of protein to load on the gel and the best dilutions of primary and secondary antibodies must be determined empirically. Some common questions are addressed below:

Problem	Cause
High background	□ Reduce primary antibody concentration by increasing the dilution factor. □ Try a different blocking buffer. □ Try a shorter exposure time. □ Increase washing time.
No or low signal	□ Check that correct primary antibody used. □ Check that secondary antibody recognizes primary (for example if the primary is a rabbit antibody, that the secondary is goat-anti-rabbit).

Problem	Cause
White spots within bands	Improve transfer, making sure to remove any bubbles between the gel and the membrane
Speckled background	Filter secondary antibody. Filter blocking and washing buffers. Ensure that the laboratory environment is clean, to minimize dust, debris or any other particles that might come in contact with the blot. Cover the dish during incubation or washing steps. Use non-powdered gloves, or switch to a different kind of gloves. We recommend powder-free nitrile gloves or polyethylene gloves.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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