

Product Information

ProteoQwest™ Chemiluminescent Western Blotting Kit, CPS Substrate

For Mouse Monoclonal IgG Antibodies

Catalog Number **PQ0201**

Store at 2–8 °C

TECHNICAL BULLETIN

Product Description

The ProteoQwest Chemiluminescent Western Blotting Kit, CPS Substrate includes essential reagents and antibodies used in a Western blot specific for mouse monoclonal IgG antibodies. This ProteoQwest kit is designed for high sensitivity chemiluminescent detection of as little as 0.03 ng of protein. The chemiluminescent reaction occurs directly on the protein immobilized membrane.¹ This kit is optimized for use with either film or chemiluminescent imagers.

The Chemichrome™ Western Control is a positive control used throughout the entire Western blotting process. The Western Control is designed for qualitative determination in Laemmli SDS-PAGE systems, and for use as a visual check of Western transfer efficiency. Mouse IgG has been added to the Chemichrome Western Control as a positive control. The heavy chain (50 kDa) of the Mouse IgG will be detected using the anti-mouse secondary antibody supplied with this kit. For more details on the Chemichrome Western Control, please see the Chemichrome Western Control Technical Bulletin.

All components of the ProteoQwest Kit have been extensively tested and optimized. This kit is designed for 12 mini-gel sized (10 cm × 10 cm) blots.

Components

- 200 µl vial of Chemichrome Western Control (Catalog Number C2242)
- 400 ml bottle of Western Blocker Solution (Catalog Number W0138)
- 12 packets each to prepare 500 ml of Tris Buffered Saline with 0.05% TWEEN[®] 20, pH 8, (TBST, Catalog Number T9447)

- 75 µl vial of Rabbit Anti-Mouse IgG (whole molecule) Horse Radish Peroxidase conjugate antibody (Catalog Number A5225)
- 80 ml bottle of Chemiluminescent Reaction Buffer (Catalog Number C9232)
- 40 ml bottle of Chemiluminescent Reagent (Catalog Number C9107)

Reagents and Equipment Required but Not Provided

- SDS-PAGE gels, running buffer (Catalog Number T7777), and gel unit or apparatus
- Nitrocellulose (Catalog Number N5891) or PVDF (Catalog Number P4188) membranes
- Blotting Paper (Catalog Number P7796), Western Transfer Buffer (Catalog Number T4904), Methanol (Catalog Number M1775), and a Western blotting apparatus
- Primary mouse monoclonal IgG antibody specific to protein of interest
- Kodak BioMax ML-2 film for capturing chemiluminescent data (Catalog Number Z370428)
- Kodak Developer (Catalog Number P7042)
- Kodak Fixer (Catalog Number P7167)
- Film Cassette

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

- Immediately before use, reconstitute Tris Buffered Saline with 0.05% TWEEN 20 (TBST, Catalog Number T9447) using 500 ml of ultrapure water (18 MΩ·cm or equivalent). When filtered into a sterile container with a sterile 0.2 µm filter, the solution is stable for 2 weeks at 2–8 °C.

Storage/Stability

The ProteoQwest Chemiluminescent Western Blotting Kit and the Chemichrome Western Control come in two separate packages. Store the kit at 2–8 °C, store the Chemichrome Western Control at –20 °C. All of the components are stable for at least 1 year if stored at suggested temperatures.

Procedure

Each researcher must optimize the Western blotting system for the protein of interest. Use the recommended amount of each reagent and antibody in the procedure below and then optimize the system as needed (see Optimization Tips). The Chemichrome Western Control should be used as a control in every blot, even after optimization.

I. Western Blotting Detection

The procedure below is designed for 12 mini-gel sized blots. All incubation and wash steps should be done in a clean container, at room temperature, with slight agitation.

1. Load 5 μ l of the Chemichrome Western Control (Catalog Number C2242) into a protein gel system of choice.
2. Run the gel and transfer it to a membrane (Nitrocellulose or PVDF). Use the colored bands of the Chemichrome to verify that the proteins have transferred to the membrane (see Table 1 for apparent molecular masses).
3. Wash the membrane for 1 minute with TBST.
4. Place the membrane in a container with at least 15 ml of Western Blocker Solution (Catalog Number W0138). Make sure there is enough Western Blocker Solution to cover the membrane. Incubate for 30 minutes.
5. A mouse monoclonal IgG antibody must be used as the primary antibody with this kit. Pipette 10 μ g of primary antibody per ml of blocker into the blocker solution from step 4. The primary antibody is specific to the protein of interest. See Optimization Tip 1. Incubate for 30 minutes, then discard the solution.
6. Wash the membrane with TBST for 1 minute. See Optimization Tip 2. After the minute incubation, discard the TBST.
7. Add at least 15 ml of fresh Western Blocker Solution to the membrane.
8. Make a 1:300,000 dilution of anti-mouse secondary antibody conjugate (Catalog Number A5225) with the Western Blocker solution from step 7. See Optimization Tip 3. Incubate for 30 minutes, then discard the solution.
9. Wash the membrane 5 times for 5 minutes each time with TBST. See Optimization Tip 2.
10. Remove the membrane from the wash buffer and drain any excess liquid from the membrane. Keep the membrane damp. Do not let the membrane dry out.
11. Place the membrane on a flat sheet of plastic wrap or on any clean plastic surface.
12. Use the substrate ratio that corresponds to the type of detection system being used, either a film based system or a chemiluminescent imaging based system.

Film Based System

1. Make enough Chemiluminescent Peroxidase Substrate to cover each membrane. Typically at least 5 ml of substrate is used on a mini-gel sized membrane. For film, make fresh Chemiluminescent CPS Substrate by lightly vortexing 1 ml of Chemiluminescent Reagent (Catalog Number C9107) per 2 ml of Chemiluminescent Reaction Buffer (Catalog Number C9232).
2. Incubate the membrane with Chemiluminescent CPS Substrate at room temperature for 5 minutes. See Optimization Tip 4.
3. Remove the excess substrate from the membrane and enclose it in a clean plastic sheet. This is done so that the film has no contact with the substrate.
4. Place the plastic sheet with the membrane in a film exposure cassette. Expose the membrane in a dark room to chemiluminescent compatible film for 30 seconds. See Optimization Tip 5.
5. Place the exposed film in developer for a length of time (developer dependent). Rinse the film in ultrapure water.
6. Place the film in fixer for a length of time (fixer dependent). Rinse the film in ultrapure water.
7. The heavy chain (50 kDa) of the Mouse IgG in the Chemichrome Marker Control should now be visible on the film. See the Optimization Tips Section or the Troubleshooting Guide if results are not as desired.

Chemiluminescent Imaging System

To achieve a digital picture with high sensitivity and high resolution, the exposure time may take up to 8 times longer than a traditional dark room/film based system.

1. Make enough Chemiluminescent Peroxidase Substrate to cover each membrane. Typically at least 5 ml of substrate is used on a mini-gel sized membrane. For an imaging system, make fresh Chemiluminescent Peroxidase Substrate by lightly vortexing 1 ml of Chemiluminescent Reagent (Catalog Number C9107) per 1 ml of Chemiluminescent Reaction Buffer (Catalog Number C9232).
2. Incubate the membrane with the Chemiluminescent Peroxidase Substrate at room temperature for 5 minutes. See Optimization Tip 4.
3. Remove the excess substrate from the membrane and place it on a clean plastic sheet.
4. Place the plastic sheet with the membrane on the tray in the imager. Set up the imager to detect chemiluminescent signals and expose the membrane for the amount of time needed to detect the protein of interest. See the Optimization Tips Section or the Troubleshooting Guide if results are not as desired.

II. Optimization Tips

The following tips should be followed when trying to optimize this kit's procedure for the detection of the protein of interest.

1. The amount of primary antibody (0.1-20.0 $\mu\text{g/ml}$) will have to be optimized for each protein of interest. It is suggested that a concentration of 10 $\mu\text{g/ml}$ is used first and then decreased or increased as necessary.
2. The number of TBST washes after the primary and secondary antibody incubations affects nonspecific binding. Increase the number of washes after each incubation if needed.
3. The amount of anti-mouse secondary antibody conjugate (1:80,000 to 1:500,000) will have to be optimized for each protein of interest. It is suggested that a dilution of 1:300,000 is used first and then decreased or increased as necessary.

4. It is possible to decrease or increase the chemiluminescent signal by incubating the membrane with Chemiluminescent Peroxidase Substrate for 1 to 20 minutes, instead of the recommended 5 minutes.
5. To increase or decrease the chemiluminescent signal captured onto film, expose the film to the membrane for more than or less than the recommended 30 seconds.
6. Gloves must be worn at all times when handling membranes (Nitrocellulose, PVDF) to avoid protein contamination of membranes.
7. Do not place azide in any buffer solution for it inhibits Horseradish Peroxidase (HRP).

Table 1.

Apparent Molecular Masses (kDa) of Proteins in Chemichrome Western Control

Band Color	4–20% Gel Tris-Glycine	10–20% Gel Tris-Tricine
Violet	220	210
Pink	100	90
Blue	60	65
Pink	45	40
Orange	30	30
Blue	20	20
Pink	12	13
Blue	8	8

Apparent molecular weights were determined by using SigmaMarker™, Wide Range (6.5-205 kDa) as a standard. The molecular weight of the violet band, which is outside the range of the standard, is an approximation.

Troubleshooting Guide

It is best to do a dot blot before doing your first Western blot to make sure that the amount of each antibody is correct. Below are some common problems and their corresponding solutions.

Problem	Cause	Solution
Too much background signal observed on film or imager.	Not enough wash steps at the end of the blotting	Double the number of washing steps.
	Too much primary antibody used.	Decrease the amount of primary antibody used and wash with TBST for 5 minutes instead of 1 after the primary antibody incubation.
	Too much secondary antibody used.	Decrease the amount of secondary antibody used.
Nonspecific bands show up on film or imager.	Too much primary antibody used.	Decrease the amount of primary antibody used and wash with TBST for 5 minutes instead of 1 after the primary antibody incubation.
	Too much secondary antibody used.	Decrease the amount of secondary antibody used.
Chemiluminescent signal does not last for a long period of time.	Signal degrades over time.	For a longer duration signal make the Chemiluminescent Peroxidase Substrate a 1:1 ratio of Chemiluminescent Reaction Buffer to Chemiluminescent Reagent. After the signal is no longer detectable, reapply fresh substrate to membrane.
No chemiluminescent signal is detected on film or imager.	Low amounts of specific protein present.	Expose the membrane to film or imager for a longer period of time.
	Insufficient primary antibody used.	Use more primary antibody.
	Insufficient secondary antibody used.	Use more secondary antibody.
	Protein degraded into fragments.	Add protease inhibitors to original sample before running a gel.
No color marker proteins seen on membrane.	Transferred in the wrong direction.	Re-run gel and transfer again.
	Did not transfer long enough.	Reassemble blotting apparatus and continue transfer.
No heavy chain of mouse IgG detected on film or imager.	Insufficient secondary antibody used.	Use a 1:80,000 to 1:500,000 dilution of secondary antibody to detect the heavy chain of Mouse IgG.
	Did not let the Chemiluminescent Peroxidase Substrate stay on membrane long enough.	Let the substrate stay on the membrane for at least 5 minutes.
	Did not expose film or imager to membrane long enough.	Keep doubling the exposure time until heavy chain is detected.

References

- Harlow, E., and Lane, D., Antibodies: A Laboratory Manual. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1988).

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