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ProductInformation

ProteoQwest ä Colorimetric Western Blotting Kit, TMB Substrate
For Mouse Monoclonal IgG Antibodies

Product Code **PQ0101**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

ProteoQwest Colorimetric Western Blotting Kit, TMB Substrate includes essential reagents and antibodies used in a Western blot specific for mouse monoclonal IgG antibodies. This ProteoQwest Colorimetric kit is designed for high sensitivity colorimetric detection. The colorimetric reaction occurs directly on the protein immobilized membrane.[1] No dark room or film is needed when performing a Western blot with this ProteoQwest Colorimetric kit. This kit has similar sensitivity levels to chemiluminescent detection systems; it can detect as little as 0.15 ng.

One of the most important components of this kit is the Chemichrome Western Control. The Chemichrome Western Control is used as a control 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 this ProtoeQwest kit have been extensively tested and optimized. This kit is designed for 25 mini-gel sized (10 × 10 cm) blots. It is possible to use this kit for as many as 45 blots if half the suggested amount of reagents are used.

Components

- 200 µl vial of Chemichrome Western Control (Product Code C2242)
- 2 × 400 ml bottles of Western Blocker Solution (Product Code W0138)
- 25 packets each to prepare 500 ml of Tris Buffered Saline with 0.05% TWEEN® 20 (TBST), pH 8.0 (Product Code T9447)
- 75 µl vial of Rabbit Anti-Mouse IgG (whole molecule)–Horse Radish Peroxidase conjugated antibody (Product Code A5225)
- 100 ml bottle of TMB Substrate for peroxidase detection on Membranes [3,3′,5,5′, Tetramethyl-benzidine (TMB) Liquid Substrate System for membranes (Product Code T0565)]

Reagents and Equipment Required But Not Provided

- SDS-PAGE gels, Running buffer (Product Code T7777), and gel unit or apparatus
- Nitrocellulose (Product Code N5891) or PVDF (Product Code P4188) membranes
- Blotting Paper (Product Code P7796), Western transfer buffer (Product Code T4904), Methanol (Product Code M1775), and a Western blotting apparatus
- Primary mouse monoclonal IgG antibody specific to protein of interest.

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, Product Code T9447) using 500 ml of ultrapure water (18 MΩ·cm or equivalent). When filtered into a sterile container using a sterile 0.2 µm filter, the solution is stable for 2 weeks at 2–8 °C.

Storage/Stability
The ProteoQwest Colorimetric Western Blotting Kit and the Chemichrome Western Control come in two separate packages. Upon receipt, store the kit at 2–8 °C and 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 25 mini-gel sized blots. All incubation and wash steps should be done in a clean container, at room temperature, and with slight agitation.

1. Load 5 µl of the Chemichrome Western Control (Product Code C2242) and the protein samples 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 weights).

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 (Product Code 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 µg 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 1:10,000 dilution of Anti-Mouse IgG (whole molecule)–Horse Radish Peroxidase conjugated antibody (A 5225) 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. Place enough TMB solution (Product Code T0565) on the membrane to completely cover the membrane's surface. Typically 5 ml is enough to cover a mini-gel (10 × 10 cm) size membrane.

13. Expose the membrane to the TMB solution at room temperature for 5-15 minutes. Visually monitor the reaction. Remove the substrate when protein bands are visible and the background is still low. High background diminishes the contrast between positive signal and background.

14. Wash the membrane in ultrapure water for 1 minute.

15. Store the membrane in the dark in fresh ultrapure water. If stored correctly, signal should remain on the membrane for a week. During that time, capture the membrane's image using a camera or scanner.

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 to 20.0 µg/ml) will have to be optimized for each protein of interest. It is suggested to use 10 µg/ml first and then adjust the concentration 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 dilution (1:1,000 to 1:100,000) of Anti-Mouse IgG (whole molecule)–Horse Radish Peroxidase conjugated antibody (Product Code A5225) will have to be optimized for each protein of interest. It is suggested that a dilution of 1:10,000 is used first and then decreased or increased as necessary.

4. Gloves must be worn at all times when handling membranes (Nitrocellulose, PVDF) to avoid protein contamination of membranes.

5. Do not place azide in any buffer solution for it inhibits horseradish peroxidase (HRP).

Table 1.
Apparent Molecular Weights (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 to 205 kDa) as a standard. The molecular weight of the violet band, which is outside the range of the standard, is an approximation.

References

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

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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 membrane.	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 appear on membrane.	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
Colorimetric signal does not last for a long period of time.	Signal degrades over time.	If stored correctly, signal should remain on the membrane for a week. During that time, capture the membrane's image using a camera or scanner.
No colorimetric signal observed on membrane.	Low amounts specific protein present.	Expose the membrane to film or imager for a longer period of time.
	Insufficient amount of primary antibody used.	Use more primary antibody
	Insufficient amount of secondary antibody used.	Use more secondary antibody
	Protein degraded into fragments.	Add protease inhibitors to original sample before running gel.
No color marker proteins observed 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 observed on membrane.	Insufficient amount of secondary antibody used.	Increase the concentration of secondary antibody used (i.e., decrease the dilution of secondary antibody).
	Did not let the TMB substrate stay on membrane long enough.	Let the substrate stay on the membrane for at least 5 minutes.