

## Product Information

### **ProteoQwest™ Colorimetric Western Blotting Kit, BCIP®/NBT Substrate**

For Mouse Monoclonal IgG Antibodies

Catalog Number **PQ0111**

Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### **Product Description**

The ProteoQwest™ Colorimetric Western Blotting Kit, BCIP®/NBT Substrate includes essential reagents and antibodies for use with Western blot specific mouse monoclonal IgG antibodies. The ProteoQwest kit is designed for low non-specific binding/background and high sensitivity colorimetric detection using an anti-mouse IgG alkaline phosphatase conjugate. The colorimetric reaction occurs directly on the membrane with immobilized protein.<sup>1</sup> No dark room or film is needed. The ProteoQwest Colorimetric kit can detect as little as 0.25 ng of immobilized target protein.

The Chemichrome™ Western Control is a positive control used throughout the entire Western blotting process. It 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. It is an ideal positive control when low levels of target protein are present. When using this BCIP/NBT substrate system, the control is used to help evaluate trouble when little or no target protein is detected. 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 25 mini-gel sized (10 cm × 10 cm) blots. It is possible to use this kit for up to 45 blots if half the suggested amounts of reagents are used.

### **Components**

- 200 µl vial of Chemichrome Western Control (Catalog Number C2242)
- 25 packets each to prepare 100 ml of Tris Buffered Saline, pH 8.0, with 3% nonfat milk (Catalog Number T8793)
- 25 packets each to prepare 500 ml of Tris Buffered Saline with TWEEN® 20 (TBST), pH 8.0 (Catalog Number T9447)
- 250 µl vial of Anti-Mouse IgG (whole molecule)–Alkaline Phosphatase antibody produced in goat (Catalog Number A3562)
- 100 ml bottle of BCIP/NBT solution, substrate for alkaline phosphatase detection on membranes (Catalog Number B6404)

### **Reagents and Equipment Required but Not Provided**

- SDS-PAGE gels, running buffer, 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

### **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 M $\Omega$ -cm or equivalent). When filtered into a sterile container using a 0.2  $\mu$ m sterile filter, the solution is stable for 2 weeks at 2-8 °C.
- Immediately before use, reconstitute Tris Buffered Saline with 3% nonfat milk (Catalog Number T8793) using 100 ml of ultrapure water. Discard after use.

### 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.

### 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 5–10  $\mu$ l of the Chemichrome Western Control (Catalog Number C2242) and experimental samples into a protein gel system of choice.
2. Electrophorese and transfer the proteins to a membrane (nitrocellulose or PVDF). Use the colored bands of the Chemichrome Western Control to verify that the proteins have transferred to the membrane (see Table 1 for apparent molecular masses).
3. Wash the membrane for 2 minutes with ultrapure water.
4. Place the membrane in a container with at least 15 ml of TBS, pH 8.0, with 3% nonfat milk (Catalog Number T8793). Make sure there is enough TBS with 3% milk to cover the membrane. Incubate for 30 minutes.
5. A mouse monoclonal IgG antibody specific to the protein of interest must be used as the primary antibody with this kit. Pipette 1–10  $\mu$ g of primary antibody per ml of blocker into the blocker solution from step 4. See Optimization Tip 1. Incubate for 30–60 minutes, then discard the solution.
6. Wash the membrane 4 times for 5 minutes each time with TBST (Catalog Number T9447). See Optimization Tip 2. After the incubation, discard the TBST.
7. Add at least 15 ml of fresh TBS with 3% nonfat milk to the membrane.
8. Make a 1:7,500 dilution of Anti-Mouse IgG (whole molecule)-Alkaline Phosphatase conjugate antibody (Catalog Number A3562) into blocker from step 7. See Optimization Tip 3. Incubate for 30–60 minutes, then discard the solution.
9. Wash the membrane 4 times for 5 minutes each time with TBST (Catalog Number T9447). See Optimization Tip 2.
10. Remove membrane from wash buffer and wash membrane in water 5 times for 2 minutes each with ultrapure water. Drain any excess liquid from the membrane. Keep the membrane damp. Do not let the membrane dry.
11. Place the membrane on a flat sheet of plastic wrap or on any clean plastic surface.
12. Use enough BCIP/NBT solution (Catalog Number B6404) to completely cover the membrane surface. Typically 4 ml is enough to cover a mini-gel (10 cm  $\times$  10 cm) size membrane.
13. Incubate the membrane with the BCIP/NBT 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.
14. Wash the membrane in ultrapure water for 5 minutes.
15. Store the membrane in the dark in fresh ultrapure water until the membrane image has been captured using a camera or scanner. See Optimization tip 4.
16. If desired, dry the membrane on filter paper for long term storage in the dark.

### 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 µg/ml) may 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. Increasing the number of TBST washes after the primary and secondary antibody incubations decreases nonspecific binding. If needed, increase the number of washes after each incubation.
3. The dilution (1:1,000 to 1:50,000) of Anti-Mouse IgG (whole molecule)-Alkaline Phosphatase (A3562) may have to be optimized for each protein of interest. It is suggested that a dilution of 1:7,500 is used first and then decreased or increased as necessary.
4. To decrease the background and to better visualize the Chemichrome IgG band, incubate the membrane in water overnight and observe the membrane the following day. A faint band of Chemichrome IgG will become more pronounced.
5. Gloves must be worn at all times when handling membranes (nitrocellulose, PVDF) to avoid protein contamination.

**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 masses were determined by using SigmaMarker™, Wide Range (6.5–205 kDa) as a standard. The molecular mass of the violet band, which is outside the range of the standard, is an approximation.

### Troubleshooting Guide

It is best to complete a dot blot before performing your first Western blot to ensure that the amount of each antibody is correct. Below are some common problems and corresponding solutions.

Problem	Cause	Solution
Too much background signal observed.	Not enough wash steps at the end of blotting	Double the amount of wash steps.
	Too much primary antibody used.	Decrease the amount of primary antibody used.
	Too much secondary antibody used.	Decrease the amount of secondary antibody used.
Nonspecific bands found on membrane.	Too much primary antibody used.	Decrease the amount of primary antibody used.
	Too much secondary antibody used.	Decrease the amount of secondary antibody used.
Colorimetric signal diminishes.	Signal degrades over time.	If stored correctly, signal should remain on the membrane for at least a week. During that time, capture the membrane's image using a camera or scanner.
No colorimetric signal observed on membrane except for the Chemichrome Mouse IgG control.	Low amounts of specific protein present.	Expose membrane to BCIP/NBT solution longer.
	Insufficient amount of primary antibody used.	Use more primary antibody.
	Insufficient amount of secondary antibody used.	Use more secondary antibody.
No color marker proteins observed on membrane.	Transferred in the wrong direction.	Re-run gel and transfer again, carefully confirming the direction of transfer and assembly of components
	Did not transfer long enough.	Reassemble blotting apparatus and continue transfer.
No heavy chain of Chemichrome Mouse IgG control is observed on the membrane, but colored markers are transferred.	Insufficient amount of secondary antibody used.	Increase the concentration of secondary antibody used.
	BCIP/NBT substrate did not stay on membrane long enough.	Let the substrate stay on the membrane for at least 5 minutes.

### References

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

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