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ProductInformation

ProteoQwest™ Colorimetric Western Blotting Kit, TMB Substrate
For Rabbit Polyclonal IgG Antibodies

Product Code **PQ0121**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The ProteoQwest™ Colorimetric Western Blotting Kit, TMB Substrate includes essential reagents and antibodies for use with Western blot specific rabbit IgG antibodies. The ProteoQwest kit is designed for low non-specific binding/background and high sensitivity colorimetric detection using anti-rabbit IgG horseradish peroxidase (HRP) conjugate. The colorimetric reaction occurs directly on the membrane with the immobilized protein.[1] No dark room or film is needed. The ProteoQwest Colorimetric kit can detect as little as 0.125 ng of immobilized target protein.

Chemichrome™ Ultimate is a positive control used throughout the entire Western blotting process. It is designed for qualitative determination in Laemmli SDS-PAGE systems[2] and for use as a visual check of Western transfer efficiency. Rabbit IgG has been added to the Chemichrome Ultimate as a positive control. The heavy chain (50 kDa) of the rabbit IgG will be detected using the anti-rabbit secondary antibody supplied with this kit. A band at ~100 kDa (heavy chain dimer) or 25 kDa (light chain) may also be detected. When using this TMB substrate system, the Chemichrome Ultimate is used to confirm suitable analysis conditions. For more details on Chemichrome Ultimate, please see the Chemichrome Ultimate Technical Bulletin.

All components of the ProteoQwest 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 up to 45 blots if half the suggested amounts of reagents are used.

Components

- 200 µl vial of Chemichrome Ultimate (Product Code C2117)
- 25 packets each to prepare 100 ml of Tris Buffered Saline, pH 8.0, with 3% nonfat milk (Product Code T8793)
- 25 packets each to prepare 500 ml of Tris Buffered Saline with TWEEN® 20 (TBST), pH 8.0 (Product Code T9447)
- 250 µl vial of Goat Anti-rabbit IgG (whole molecule) HRP conjugate antibody (Product Code A8102)
- 100 ml bottle of TMB substrate for HRP detection on membranes (Product Code T0565)

Reagents and Equipment Required But Not Provided

- SDS-PAGE gels, running buffer, 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)
- Western blotting apparatus
- Primary rabbit 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 0.2 µm sterile filter, the solution is stable for 2 weeks at 2-8 °C.
- Immediately before use, reconstitute Tris Buffered Saline, pH 8.0, with 3% nonfat milk (Product Code T8793) using 100 ml of ultrapure water. Discard after use.

Storage/Stability
The ProteoQwest Colorimetric Western Blotting Kit and Chemichrome Ultimate arrive in two separate packages. Upon receipt, store the kit at 2–8 °C and the Chemichrome Ultimate 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). Chemichrome Ultimate 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 performed in a clean container, at room temperature, and with slight agitation.**

1. Load experimental samples and 5 µl of the Chemichrome Ultimate (Product Code C2117) into a protein gel system of choice.
2. Electrophorese and transfer the proteins to a membrane (nitrocellulose or PVDF). Use the colored bands of Chemichrome Ultimate to verify that the proteins have transferred to the membrane (see Table 1 for apparent molecular masses).
3. Wash the membrane for at least 2 minutes with ultrapure water.
4. Place the membrane in a container with at least 15 ml of TBS, pH 8.0, with 3% milk (Product Code T8793). Make sure the TBS with 3% milk covers the membrane. Incubate for at least 30 minutes.
5. A rabbit IgG antibody specific to the protein of interest must be used as the primary antibody with this kit. Pipette 1 to 2.5 µg 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 (Product Code T9447). See Optimization Tip 2. After the incubation, discard the TBST.
7. Add at least 15 ml of fresh TBS with 3% milk to the membrane.
8. Dilute 1:30,000 Anti-Rabbit IgG (whole molecule) HRP conjugate antibody (Product Code A8102) in the 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 (Product Code T9447). 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.
11. Place the membrane on a flat sheet of plastic wrap or on any clean plastic surface.
12. Use enough TMB solution (Product Code T0565) to completely cover the membrane's surface. Typically 4 ml is sufficient to cover a mini-gel (10 × 10 cm) size membrane.
13. Incubate the membrane with the TMB solution at room temperature for 5-30 minutes. Visually monitor the reaction. Remove the substrate solution when protein bands are visible and the background is still low.
14. Wash the membrane in ultrapure water for 5 minutes.
15. The membrane may be stored in the dark in fresh, ultrapure water for up to a week, until the image of the membrane has been captured using a camera or scanner.
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 procedure for the detection of the protein of interest.

1. The amount of primary antibody (0.1 to 20.0 µg/ml) may need to be optimized for each protein of interest. Use 1 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 (1:8,000 to 1:200,000) of Anti-Rabbit IgG (whole molecule) **–** HRP (Product Code A8102) may need to be optimized for each protein of interest. It is suggested that a dilution of 1:30,000 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.

Table 1.
Apparent Molecular Masses (kDa) of Proteins in Chemichrome Ultimate

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 number 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.
Decrease in colorimetric signal with time	Signal degrades over time.	If stored correctly, signal should remain on the membrane for at least a week. During that time, capture the image of the membrane using a camera or scanner.
No colorimetric signal observed on membrane except for the Chemichrome Ultimate Rabbit IgG control.	Low amounts of specific protein present.	Expose membrane to TMB 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 Ultimate Rabbit 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.
	TMB substrate did not stay on membrane long enough.	Let the substrate stay on the membrane for at least 5 minutes.

References

1. Harlow, E., and Lane, D., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (Plainview, NY: 1988).
2. Laemmli, U.K., Nature, **227**, 680-685 (1970).

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