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Product Information

ProteoQwest™ Chemiluminescent Western Blotting Kit, CPS Substrate
for Rabbit Polyclonal IgG Antibodies

Catalog Number **PQ0221**

TECHNICAL BULLETIN

Product Description

The ProteoQwest™ Chemiluminescent Western Blotting Kit, CPS 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 chemiluminescent detection using an anti-rabbit IgG horseradish peroxidase (HRP) conjugate. The ProteoQwest Chemiluminescent kit can detect as little as 0.06 ng of immobilized target protein.

ColorBurst™ Electrophoresis Marker is a positive control used throughout the entire Western blotting process. It is designed for qualitative determination in Laemmli SDS-PAGE systems[1] and for use as a visual check of Western transfer efficiency.

This kit is designed for 12 mini-gel sized (10 cm × 10 cm) blots. It is possible to use this kit for up to 24 blots if half the suggested amounts of reagents are used.

Components

- 500 µl vial of ColorBurst Electrophoresis Marker (Catalog Number C1992)
- 12 packets each to prepare 100 ml of Tris Buffered Saline, pH 8.0, with 3% nonfat milk (Catalog Number T8793)
- 12 packets each to prepare 500 ml of Tris Buffered Saline with 0.05% TWEEN® 20 (TBST), pH 8.0, (Catalog Number T9447)
- 250 µl vial of Goat Anti-rabbit IgG (whole molecule) HRP conjugate antibody (Catalog Number A8102)
- 60 ml of CPS-1 substrate for HRP detection on membranes (Catalog Number CPS160) including 20 ml Chemiluminescent Reagent (Catalog Number C9107) and 40 ml Chemiluminescent Reaction Buffer (Catalog Number C9232).

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)
- 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, Catalog Number 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 with 3% nonfat milk (Catalog Number T8793) using 100 ml of ultrapure water. Discard after use.

Storage/Stability

The ProteoQwest Chemiluminescent Western Blotting Kit and ColorBurst Electrophoresis Marker arrive in two separate packages. Upon receipt, store the kit at 2–8 °C and store the ColorBurst Electrophoresis Marker 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 then optimize the system as needed (see Optimization Tips). The ColorBurst Electrophoresis Marker should be used as a control in every blot, even after optimization.

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

1. Load experimental samples and 5 µl of the ColorBurst Electrophoresis Marker (Catalog Number C1992) 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 ColorBurst Electrophoresis Marker to verify 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% nonfat milk (Catalog Number T8793). Make sure that TBS with 3% nonfat 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 1–2.5 µg of primary antibody per ml of blocker solution 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. Dilute 1:80,000 Anti-Rabbit IgG (whole molecule) HRP conjugate antibody (Catalog Number A8102) in blocker solution 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 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. Prepare CPS-1 Solution. Do a 2:1 dilution, add 2 ml of Chemiluminescent Reaction Buffer (Catalog Number C9232) to 1 ml of Chemiluminescent Reagent (Catalog Number C9107).
13. Use enough of the prepared CPS-1 solution to completely cover the surface of the membrane. Typically 4 ml is enough to cover a mini-gel (10 cm × 10 cm) size membrane.
14. Incubate the membrane with the prepared CPS-1 solution at room temperature for 5 minutes.
15. Develop in dark room or on a chemiluminescent scanner.

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 (1:50,00 to 1:500,000) of Anti-Rabbit IgG (whole molecule) HRP (Catalog Number A8102) may need to be optimized for each protein of interest. It is suggested that a dilution of 1:80,000 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 ColorBurst Electrophoresis Marker

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.
No chemiluminescent signal observed on membranel.	Low amounts of specific protein present.	Expose membrane to CPS-1 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.

References

1. Laemmli, U.K., Nature, **227**, 680-685 (1970).

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