Product Information

ProteoQwest™ FLAG® Colorimetric Western Blotting Kit, TMB Substrate
For colorimetric detection of FLAG-tagged proteins

Catalog Number PQ0300

Technical Bulletin

Product Description
ProteoQwest FLAG Colorimetric Western Blotting Kit, TMB Substrate, includes a FLAG-tagged control protein, Monoclonal ANTI-FLAG® M2–Peroxidase, and liquid 3,3',5,5'-tetramethylbenzidine (TMB) substrate for direct colorimetric detection of FLAG epitope-tagged proteins on Western blots.

The use of the Monoclonal ANTI-FLAG M2–Peroxidase for direct immunostaining eliminates non-specific binding/background and simplifies the procedure compared to the use of unconjugated ANTI-FLAG antibodies with anti-mouse IgG secondary antibody peroxidase conjugates. Superior results can be obtained for immunostaining blots from immunoprecipitation experiments because there is no reaction between the ANTI-FLAG M2–Peroxidase and the heavy and light chains of the antibody used for immunoprecipitation. In addition, the colorimetric reaction occurs directly on the membrane and can be visually monitored for desired signal strength. Neither a dark room nor film is required.

The ProteoQwest FLAG Colorimetric Western Blotting Kit can detect as little as 1 ng of FLAG-tagged target protein per lane on a standard 12 well mini-gel blot. This kit contains reagents sufficient for immunostaining 25 mini-gel (10 x 10 cm) blots.

Storage/Stability
The ProteoQwest FLAG Colorimetric Western Blotting Kit, TMB Substrate, arrives in two separate packages—one containing the two components of Part A, bundled and labeled as Catalog No. PQ0301, and one containing Part B, Catalog No. T0565. Upon receipt, store the TMB Liquid Substrate System for Membranes at 2-8 °C and the Amino-terminal FLAG-BAP Fusion Protein and ANTI-FLAG M2–Peroxidase at ~20 °C. All of the components are stable for at least one year when stored properly.

Components-Part A and Part B shipped separately

Part A - Labeled as Catalog No. PQ0301
Amino-terminal FLAG-BAP Fusion Protein 100 µg
Catalog No. P7582
Solution in 10 mM Tris, pH 8.0, 120 mM NaCl, 0.05 mM ZnCl₂ in 50% glycerol. The control protein has a calculated molecular weight of 49.3 kDa.

Monoclonal ANTI-FLAG M2–Peroxidase 1 mg
Catalog No. A8592
Solution in 10 mM sodium phosphate, pH 7.4, 150 mM NaCl, containing 50% glycerol plus stabilizer and preservative. The conjugate protein concentration is ~1 mg/ml.

Part B
3,3',5,5'-Tetramethylbenzidine (TMB) 100 ml
Liquid Substrate System for Membranes, Catalog No. T0565

Reagents and Equipment Required But Not Provided
- SDS-PAGE gels, running buffer, and gel unit or apparatus
- Sample Buffer, Laemmli Buffer, 2x Concentrate, Catalog No. S3401
- ColorBurst™ Electrophoresis Markers, Catalog No. C1992
- Transfer membranes - Nitrocellulose; Nylon, or PVDF
- Blotting Paper, Catalog No. P7796
- Western transfer buffer, Catalog No. T4904
- Methanol, Catalog No. M1775
- Western blotting apparatus
- Ponceau S Solution, Catalog No. P7170
- Tris Buffered Saline (TBS), pH 8.0, with 3% milk, Catalog No. T8793, for blocking
- Tris Buffered Saline with TWEEN® 20 (TBST), pH 8.0, Catalog No. T9039
Precautions and Disclaimer
This product is for laboratory research use only, not for drug, household, or other uses. Consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure
There are many different procedures and variations for performing Western blotting experiments. See Reference 1 for additional information and procedures.

The following procedure is a suggested, generic method for detection of FLAG-tagged fusion proteins by immunostaining Western blots and may not be appropriate for all situations. Researchers may need to optimize the Western blotting system for the protein of interest. The Amino-terminal FLAG-BAP Fusion Protein should be run on gels and blotted as a positive control for this procedure.

1. Run protein samples on a protein gel system of choice. For a standard 12 well mini-gel, in one lane load 5 to 50 ng of the Amino-terminal FLAG-BAP Fusion Protein as a positive control, e.g., load 10 µL of the control protein diluted to 5 ng/µL. The concentration of the Amino-terminal FLAG-BAP Fusion Protein may be obtained from the lot-specific Certificate of Analysis. Prepare the control protein solution by diluting an aliquot of the Amino-terminal FLAG-BAP Fusion Protein in appropriate 1x protein gel sample buffer. Stained protein standards such as ColorBurst Markers can also be run as controls to visually monitor electrophoresis and subsequent transfer efficiency.

2. After electrophoresis, transfer the proteins from the gel to a blotting membrane of choice (nitrocellulose, nylon, or PVDF) using an appropriate Western blotting system. After the transfer is complete, the blotting membrane containing the transferred proteins can be immunostained immediately, reversibly stained with Ponceau S, or allowed to dry for storage before immunostaining.

3. Prior to immunostaining, block the unoccupied protein binding sites on the membrane. Place the membrane, protein side up, in a suitable container containing 50 to 100 ml of a blocking solution, such as 3% nonfat milk in TBS, Catalog No. T8793, so that the solution completely covers the surface of the membrane. Incubate for 30 to 60 minutes.

4. Pour off and discard the blocking solution. Immediately add 25 to 50 ml of a 1/1000 dilution of Monoclonal ANTI-FLAG M2–Peroxidase, Catalog No. A8592, diluted in fresh blocking solution. Incubate for 30 to 60 minutes.

5. Pour off and discard the diluted antibody solution and wash the membrane at least 3 times for 5 minutes with 50 ml of TBST, Catalog No. T9039, per wash.

6. Pour off the last wash and drain well. Immediately add 4 ml of the TMB Liquid Substrate solution, Catalog No. T0565, by gently pipetting it onto the surface of the blot to cover it completely. Incubate the membrane with the TMB Liquid Substrate solution without agitation for 1 to 30 minutes. Visually monitor the reaction. Stop the reaction when protein bands are clearly visible and the background is low.

7. To stop the reaction, gently pour off the TMB Liquid Substrate solution and gently wash the membrane several times with ultrapure water. The membrane may be stored in the dark in fresh, ultrapure water for up to a week or dried on blotting paper for long-term storage in the dark. The image of the immunostained membrane can be captured using a camera or scanner.

Note: Gloves should be worn when handling blotting membranes to avoid protein contamination.

The following procedure is designed for one standard mini-gel sized blot. All incubation and wash steps should be performed at room temperature in a clean container with gentle agitation, unless indicated otherwise.

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## Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too much background signal observed.</td>
<td>Insufficient blocking</td>
<td>Use suggested blocking procedure. Do not wash between blocking and Monoclonal ANTI-FLAG M2–Peroxidase incubation steps.</td>
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<tr>
<td></td>
<td>Not enough wash steps after Monoclonal ANTI-FLAG M2–Peroxidase incubation.</td>
<td>Double the number of wash steps.</td>
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<tr>
<td></td>
<td>Too much Monoclonal ANTI-FLAG M2–Peroxidase used.</td>
<td>Use more dilute Monoclonal ANTI-FLAG M2–Peroxidase.</td>
</tr>
<tr>
<td>Nonspecific bands found on membrane.</td>
<td>Too much Monoclonal ANTI-FLAG M2–Peroxidase used.</td>
<td>Use more dilute Monoclonal ANTI-FLAG M2–Peroxidase.</td>
</tr>
<tr>
<td>Decrease in colorimetric signal with time</td>
<td>Signal degrades over time.</td>
<td>Immediately capture the image of the membrane using a camera or scanner after immunostaining. Protect stained blot from light during storage.</td>
</tr>
<tr>
<td>No colorimetric signal observed on membrane except for the N-FLAG-BAP control protein.</td>
<td>Low amounts of specific protein present.</td>
<td>Expose membrane to TMB Liquid Substrate solution longer or try a more sensitive detection substrate, such as Chemiluminescent Peroxidase Substrate, Catalog No. CPS160.</td>
</tr>
<tr>
<td></td>
<td>Insufficient amount of Monoclonal ANTI-FLAG M2–Peroxidase used.</td>
<td>Use a more concentrated Monoclonal ANTI-FLAG M2–Peroxidase dilution.</td>
</tr>
<tr>
<td>No ColorBurst stained marker proteins observed on membrane.</td>
<td>Transferred in the wrong direction.</td>
<td>Re-run gel and transfer again, carefully confirming the direction of transfer and assembly of components.</td>
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<tr>
<td></td>
<td>Did not transfer long enough.</td>
<td>Reassemble blotting apparatus and continue transfer.</td>
</tr>
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<td></td>
<td>Protein transferred completely through membrane.</td>
<td>Transfer for a shorter time.</td>
</tr>
<tr>
<td>Color of part of immunostained band is missing.</td>
<td>Poor contact between gel and membrane.</td>
<td>Remove any particles or air bubbles trapped between the blot and the membrane that might inhibit transfer. Do not touch gel or membrane with bare hands during procedure.</td>
</tr>
<tr>
<td></td>
<td>Substrate precipitate disturbed or removed.</td>
<td>Handle blot carefully after color development, so as not to inadvertently physically remove or scrape substrate precipitate off of the blotting membrane.</td>
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## References


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