ITSI BIOSCIENCES
FAST, ACCURATE, EASY- TO-USE

w-BLOT pre-blocked western blot

IMPORTANT: K-0045 is pre-blocked western blot on Nitrocellulose[1] membrane containing 9 protein samples. The 10th lane is 3ng of a protein that binds IgG. This can be used as a positive control to ensure your detection system is working. And it can also be used to normalize multiple blots in an experiment using the same antibody.

Attributes:

- High sensitivity
- High quality of proteins
- Normalized to amount of protein used in blots and arrays
- Interblot control built into every blot
- Broad selection of tissues
- Suitable for both radioactive and non-radioactive detection

STORAGE: Blot should be stored at 4°C upon receipt.

Read the procedure completely and assemble all materials needed before starting.

MATERIALS PROVIDED IN THIS KIT:

Item	Size	Catalog #	Storage
wBLOT	1 membrane	K-0045-1	4°C.
Blocking agent	5 grams	K-0045-2	Rm. T.
Stained blot image	1 color blot image		
Procedure			

MATERIALS REQUIRED but Not supplied:

1. TBS+0.05% tween20 (TBS-t). recipe below
2. Plastic incubation/washing tray for wblot
3. Stripping Buffer if reprobing. recipe below

Recipes:

TBS-t

- 9g NaCl, 25ml 1M Tris-HCl (pH 7.5), 0.50ml tween 20, bring volume to 1000ml with milliQ water.

Blocking reagent

- Add 5g of included blocking agent in 200ml of TBS-t. Stir until completely dissolved. Do not shake or excess foaming will occur. If desired 0.01% of sodium azide can be added to prevent bacterial growth. **Note: sodium azide will inhibit enzymatic activity so should be washed away before any chemiluminescent assay.**

Stripping Buffer

- 2g SDS, 12.5ml of 500 mM Tris-HCl (pH 6.8), 0.7ml 2-mercaptoethanol. Bring volume to 100ml with milliQ water

PROCEDURE:

Western Procedure

1. Rehydrate and wash the blot with approximately 20 ml TBS-t for 10 minutes.
2. Incubate the blot with your primary antibody diluted in blocking reagent (the amount of antibody dilution should be determined empirically) in enough volume to cover the blot, for 2 hours at room temperature or 4°C overnight.
3. Wash the blot with about 40 ml of TBS-t for 10 minutes. Repeat for a total of 2 washes.
4. Hybridize the blot with the secondary antibody diluted in 20 ml blocking reagent for 45-120 minutes at room temperature, or 4°C overnight. Follow the secondary antibody manufactures suggestion for the incubation conditions, and the dilution amount, or determine empirically.
5. Wash the blot with 40 ml TBS-t for 10 minutes. Wash for 3x to 5x total.
6. Detect the blot.

Reprobing:

1. Rinse the membrane with TBS-t after detection, and add 50 ml stripping buffer.
2. Incubate at 50°C-65°C for 30-90 minutes. Then rinse with TBS-t.
3. Before re-probing incubate the blot in blocking reagent for 30 minutes at room temperature.

[1]PVDF is available upon request.

***CONDITIONS FOR USE OF THIS PROCEDURE/SOLUTIONS:**

This VBP is the intellectual property of ITSI Biosciences. Only complete set of reagents provided by ITSI Biosciences should be used when possible because their compatibility with the downstream application has been validated. Considering that many factors can cause experiments to fail, ITSI Biosciences cannot guarantee that the use of this VBP and solutions will lead to a successful experiment. In no event shall ITSI Biosciences be held liable for loss of samples, failure of experiments or any other damage or injury associated with the use of this procedure or associated materials and reagents.

General Safety Information:

Consider all chemicals as potentially hazardous. Only trained laboratory personnel familiar with good laboratory practice should handle this product. Protective clothing should be worn. Use caution to avoid contact with skin and eyes. If contact should occur, wash immediately with water and follow established guidelines/procedures in your laboratory. **WARNING: Intended for research use only, not for use in human, therapeutic or diagnostic applications. The end user is responsible for all local, state and federal regulations associated with the use and disposal of laboratory reagents.**

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