Ready to Use Western Blots & Protein Arrays

Features:

- 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 a variety of tissues
- Suitable for both radioactive and non-radioactive detection
- No block needed

Protocol for Western Analysis of Protein Array

Material:
1XTTBS : 4.5 g NaCl, 12.5 ml 1M Tris-HCl (pH 7.5), 0.5 ml tween 20, add water to 500 ml.
Blocking solution: 2.5 g nonfat dry milk dissolved in 50 ml 1XTTBS.
Procedure:
1: Wash the blot **or the array** with 20 ml 1XTTBS for 10 minutes.
2: Hybridize the blot **or the array** with the primary antibody in 20 ml Blocking solution for 2 hours at room temperature or 4°C over night.
3: Wash the blot or the array with 40 ml 1XTTBS for 10 minutes twice.
4: Hybridize the blot **or the array** with 2nd antibody in 20 ml blocking solution for 45-120 minutes at room temperature or 4°C over night.
5: Wash the blot **or the array** with 40 ml 1XTTBS for 10 minutes twice.
6: Go for ECL detection.

Stripping and Reprobing

Stripping:
Stripping Buffer 1: 2% (w/v) SDS , 62.5 mM Tris-HCl (pH 6.8 at 20°C), 100 mM b -mercaptoethanol.
Stripping Buffer 2: 0.2 M Glycine-HCl (pH2.5), 0.05% Tween-20.
Method 1: Rinse the membrane with 1X TTBS after ECL detection, and add 50 ml stripping buffer 1 and incubate at 50°C-65℃ for 30-90 minutes. Then rinse with 1X TTBS again.
Note: The temperature and time of incubation depend on the interaction between antibodies and proteins, strong interactions need higher temperature and longer incubation.
Method 2: Rinse the membrane with 1X TTBS after ECL detection, and add 50 ml stripping buffer 2 and incubate at room temperature for 15-30 minutes. Then rinse with 1X TTBS again.

Reprobing:
The procedure of the reprobing is the same as the above western analysis procedure **Except Blocking the blot with 50 ml blocking buffer at room temperature for 20 minutes before adding primary antibody.**

Note: It will be easy to identify the expression signal if the size of the second target protein is dramatic different from the first one. In this case, a successfully stripping of second antibody is good enough for reprobing. Do the ECL detection right after stripping the blot or the array, no signal indicates complete stripping of the second antibody. It is crucial to strip the primary antibody completely if the size of the second protein, which is going to be detected, is the same or very similar as the first one. In order to test if the blot or the array is stripped completely, repeat step 4-6 in the western analysis procedure, if there is still strong signal, then strip the blot again.