WebSite www.ebiocell.com
E-mall order@ebiocell.com
tech@ebiocell.com

Western Blotting Protocol

This western blot protocol should be used as a guide for each researcher to build their own protocol, as each reagent will need to be optimized for use in the particular species, tissue type and application combination.

Materials

1) 1×TBST：1×TBS, 0.1% Tween 20.
2) Skim Milk .
3) Blocking Buffer：1×TBST+5% w/v Skim Milk (2g Skim Milk +40 mL 1×TBST，Mixing)；
4) Blotting Membrane：0.2uM Nitrocellulose film
5) Detection Reagent：West Femto(Strong)/West Pico(Weak)，1:1 Mixture, Usually 1mL+1Ml.
6) Developing solution (yellow), Fixing Solution (Clear, with yellow precipitation).
7) Stripping Buffer.

Procedure

Preparation Of Electrophoresis Samples

1) Configuration Reaction：Total Volume of 35ul

Protein	Water	4×LDS	β-mercaptoethanol (5%)
40μg，Calculate the volume by concentration	35μl	8.75μl	1.8μl
80μg，Calculate the volume by concentration	70μl	17.5μl	3.6μl

2) Oscillating mixing, Centrifugation.
3) Place the sample in the heat block and heat it at 100℃ for 7 minutes, Then place it on the ice to cool.
4) Oscillating mixing, Centrifugation.

Electrophoresis

1) Dilute Running Buffer (MES, 20).
2) Prepare BT preset adhesive, tear off the adhesive strip, preset adhesive is stuck in a fixed position with the high side facing forward, and a control plate is added on the back. The white press plate is pressed forward, and the "Running Buffer" is added. The bottom is over the position of the adhesive strip 1cm, and the comb is slowly pulled out vertically.
3) Sample: add 10ul "PageRuler Prestained Protein Ladder" to the first or other lane, add 30ul sample to each hole of 10 well gel sample.
4) Running: 200V, 30 minutes.

The Transfer Film

1) Preparation of Transfer Buffer: 25mL 20×"Transfer Buffer"+50mL methanol +water to fill up 500Ml.
2) Cut the NC film according to the size of the filter paper, soak it in a shaker in pure methanol for 15 minutes, and soak 50mL"Transfer Buffer" at 4℃ for later use.
3) Dip 6 layers of filter paper and 2 layers of sponge into the Transfer Buffer and precool at 4℃.
4) Prepare the film transfer according to sandwich mode (see video) : sponge + filter paper 1+ filter paper 2+ filter paper 3, pay attention to each layer of filter paper with glass rod to remove bubbles.
5) Remove the rubber plate from the electrophoresis tank and rinse with clean water;
6) Plate cutter pry open the outer plate of cutting glue.
7) Cut the glue and retain the range: the lower edge of the comb tooth to the original white glue strip.
8) Packing glue (see video).
9) Cover with NC film + filter paper 4+ filter paper 5+ filter paper 6+ sponge 2 (sandwich top).
10) Transfer the whole sandwich into the slot and clamp it into the film transfer slot.
11) Pour the Transfer Buffer in the middle over the top of the sandwich, and pour ice water around it.
12) Transfer: 35V, 1 hour; The film transfer process is best carried out in an ice box.

Sealing And Primary Antibody Incubation

1) Making blocking buffer, 5% milk.

2) After the film transfer is completed, take out the film transfer box, remove the sponge and filter paper in order, gently clamp the film and put in 20mL TBST.
3) Shake the table and wash the film at room temperature for 5 minutes (when washing the film, the adhesive side is up, and all sides are trimmed along the edge to the size of the incubator box).
4) Incubate at room temperature with 20 mL blocking buffer on shaker for 1 hour.
5) Prepare 10 mL of primary antibody solution (refer to the instructions for dilution concentration).
6) After sealing, change to primary antibody solution, and incubate overnight at 4℃ (322 temperature box, slightly shaken by shaking table.