Cut membrane, loading sample, blocking

1. According to the amount of sample to cut 8 cm $\times$ 6 cm nitrocellulose membrane. Gently draw on rectilinear reference lines per 1cm to separate the membrane into 48 grids, then marked with numbers.

2. Dilute the peptide into 5 ug/mL by 2 mL PBS (pH 7.4). Drop 2 uL the dilution slowly to the centre of the grid. Adding sample order: the upper row is NP-pep, the nether row is P-pep. Each antigen should be loaded two grids. Place 30 min to dry.

3. According to the number to cut the membrane as a unit with two grids. Incubate the membrane in blocking buffer (5% non-fat milk in TBST (m/v)) for 1 hour at room temperature.

Antibody dilution

The antibody should be diluted into 0.5 ug/mL by the blocking buffer.

Incubation primary antibody

Put the blocking membrane into the diluted antibody solution for 1 hour at room temperature.

Incubation secondary antibody

(HRP labeled goat anti rabbit Ig antibody)

1. Washing the membrane with TBST (pH 7.4) for 3 $\times$ 5min.

2. Dilute the secondary antibody with the blocking buffer.

3. Incubation secondary antibody for 1 hour at room temperature.

Coloration and developing

1. Washing the membrane with TBST (pH 7.4) for 3 $\times$ 5min.

2. Put the membrane into the substrate of HRP solution for coloration for 7 min.

3. Developing