EMSA Protocol

(For scientific research purposes only, not for clinical diagnosis!)

Histone extraction protocol for western blot

Use Nuclear protein and cytoplasmic protein extraction kit

1.1 Prepare the reagent: Dissolve the reagents at room temperature, then mix them up and put them on ice. The Nuclear protein and cytoplasmic protein extraction reagent A and B should be mixed up with PMSF until its final concentration comes to 1mM.

1.2 Adherent cells: Wash the cells three times with ice-cold PBS or EDTA. Centrifuge and collect the cells, discard the supernatant.

1.3 Suspension cells: Wash the cells three times with ice-cold PBS or EDTA. Centrifuge and collect the cells, discard the supernatant.

1.4 From fresh tissues: Cut the tissue into very small pieces as much as possible. Mix the appropriate amount of cytoplasmic protein extract A and B at a ratio of 20:1 (e.g., add 10 microliter of cytoplasmic protein extract B to 200 microliter of cytoplasmic protein extract A). Prepare a tissue homogenate with a final concentration of PMSF of 1 mM. Add 200μL of lysis buffer per 60mg tissue for machine homogenization. The homogenization should be performed in an ice bath or 4°C..Place in an ice bath for 15 minutes. After centrifugation at 2000g , 4℃ for 5 minutes, the supernatant was transferred to a precooled EP tube, which was part of the cytoplasmic protein extracted.(Never touch the sediment when supernatant.)At this stage, some plasma proteins were still not extracted, and the remaining precipitation was again extracted by the method of cell precipitation.

1.5 Add 200μL of cytoplasmic protein extraction reagent A with PMSF into each 20μL of cell precipitate.

1.6 Vortex at the highest speed for 5s, let the cell precipitates completely suspended and dispersed;(Vortex time can be extended if cell precipitation were not completely suspended and dispersed).

1.7 Maintain constant agitation for 10-15 min in ice bath.

1.8 Add 10μL Cytoplasmic protein extraction reagent B(add PMSF before use).Vortex at the highest speed for 1s. Maintain constant agitation for 1 min in ice bath.

1.9 Vortex at the highest speed for 5s,Centrifuge at 12,000-16,000g, 4°C. for 5 min.

1.10 Aspirate the supernatant(plasma protein) and place in a fresh tube kept on ice.

1.11 Aspirate the supernatant. Add 50μL nuclear protein extraction reagent B(add PMSF before use).

1.12 Vortex at the highest speed for 15-30s. Maintain constant agitation in ice bath. Vortex at the highest speed for 15-30s in between 1-2 minutes.(Total:30min).

1.13 Centrifuge at 12,000-16,000g, 4°C for 10 min.

1.14 Tranfer the supernatant to a fresh tube kept on ice. The supernatant is nuclear protein, you can immediately use or keep in -80℃ fridge.

Prepare 1.5mm native polyacrylamide gel and 0.5x TBE

Reagents	Concentration 5.5%
H_2O ml	7.50
10*TBE μl	500
40%Acrylamide ml	1.50
50% Glycerin μl	500
10%AP μl	50
TEMED μl	10
Total ml	10

Pre-electrophoresis for 60 min at 120 V .Using precool 0.5×TBE as buffer solution.

Prepare reaction mixtures as follows during pre-electrophoresis

Reagents	Negative Control μl	Sample μl	Competitor μl	Mutated competitorμl	Super Shift μl
10×binding buffer	2	2	2	2	2
1μg/μl poly（dI.dC）	1	1	1	1	1
50%glycerol	1	1	1	1	1
1%NP-40	1	1	1	1	1
100mM MgCl2	1	1	1	1	1
200mM EDTA	1	1	1	1	1
Protein	0	2	2	2	2
Labeled Probe	2	2	2	2	2
Competitor	0	0	2	0	0
Mutated competitor	0	0	0	2	0
antibody	0	0	0	0	2
DEPC	Add to 20μl				

Place at room temperature for 25-30 min。

Load the sample

After pre-electrophoresis ,change precool running buffer. Add 4 μl of 6X DNA loading buffer to sample mixture. Load the samples on gel and run the gel at 150 V for 60min.

Transfer

Put the positively charged nylon membrane into 0.5×TBE to equilibrate for 10 min. After the electrophoresis , remove the whole gel with the sample. The transferring condition is 300mA for 30min, using precool 0.5×TBE as buffer solution

Crosslinking

Place the membrane containing the reaction mixture under the UV lamp (20 cm below the light) for 20min.

Blocking

Block the membrane on a table concentrator for 20min at room temperature using blocking buffer.

Antigen-Antibody Reaction

Aspirate the blocking buffer. Incubate the membrane with appropriate dilutions of primary antibody (1:300). for 30min at room temperature.

Washing

Wash the membrane for 4 times(10min each) with 1X washing buffer.

Chemiluminescence

Take the membrane out of the eluent, absorb the liquid, put it in a chemiluminescence instrument, add ECL, wait for 1-2 minutes, absorb the excess liquid, and start chemiluminescence.

Result and analysis (shown in Excel)