

GEL SUPER-SHIFT ASSAY/ EMSA

Materials/Reagents:

See Gel-shift assay

Procedure:

1. Prepare Whole Cell Extract. Make sure every solution **DOES NOT** contain DTT.
2. Add 1 or 2 μ l of non-diluted monoclonal antibody (4B4 is better than 10H8 for HSF1) or 1/10 diluted polyclonal antibody to 10 μ g of whole cell extract from HeLa in 5 μ l of Buffer C; incubate on ice for 30 min. (Monoclonal antibody does not disturb HSF1-DNA binding activity.)

Following steps are the same as normal gel shift:

3. Add reaction mix containing 1-10 fmol (~0.1 ng) of 32 P-labeled probe and 0.5 μ g of poly(dI/dC) in 20 μ l of 1X Binding buffer. Incubate at RT for 20 min.
4. Add 2 μ l of loading dye, load total amount of the sample on 4% 0.25X TBE gel which has been pre-run for at least 20 min in 0.25X TBE buffer.
5. Electrophoresis with 100-130 V in 0.25X TBE buffer for 3 hours.
You may use 18 cm-height gel system and stop running after dye front comes to 12 cm point from the top. When a gel was run with 130 V, the current was less than 20 mA. You could run a gel with 100V. It might give better separation.

Troubleshooting/Critical Parameters:

- The reaction mixture **MUST NOT** contain DTT, because DTT kills antibody.
- Polyclonal antibody of HSF1 can be added after mixing of WCE and DNA probe. In that case, the antibody should be added in 1:100 to 1:500 final dilution.
- For Hsp70, 3A3 and C92 did work, 4G4 and 5A5 did not. 3A3 may be used 1 μ l/sample.
- HSE probe must have high-specific activity (>10,000 cpm/fmol) and be double-stranded.
- If you did not have good separation or running, you should newly prepare acrylamide and TBE stock solution.

References:

Abravaya, K. et al., *Genes & Dev.* **6**, 1153-1164, 1992
Serge, K.D. et al., *Mol. Cell Biol.*, **13**, 1392-1407, 1993

Submitted by: Gen Matsumoto