

Gel Shift/Gel Super Shift Assays

The electrophoretic gel shift assay is used to detect sequence specific DNA-binding proteins present in nuclear extracts. For NFkB a HeLa nuclear extract is used. In the assay, a consensus oligonucleotide is end-labeled with isotopic phosphorus and detected using autoradiography. Other non-radioactive methods have also been employed including chemiluminescence, fluorescence and enzymatic assays. A 'gel shift' of radiolabel is observed whenever the DNA binding protein forms a complex with radiolabeled oligonucleotide resulting in the detectable label migrating at a higher apparent molecular weight. The 'gel super shift' assay refers to the additional increase in apparent molecular weight resulting from binding a specific antibody to the DNA binding protein prior to reaction with radioactive probe. Hence through the use of a specific antibody and a consensus oligonucleotide the researcher can identify the presence of a specific DNA binding protein in any nuclear extract.

Reagents Required

Molecular Biology Grade Water. See page 74 for ordering information.

Poly d(I) d(C). Use this as a non-specific inhibitor.

1X TGE Buffer. Prepare a 10X concentrate of Tris-Glycine-EDTA (TGE) by adding 30.3 g Tris Cl, 142 g glycine, 37.2 g EDTA and deionized water to a final volume of 1.0 liter. Do not adjust the pH.

5% TGE Gel. Prepare 60 ml of solution by mixing 10.5 ml 30% polyacrylamide, 6 ml 10X TGE, 3 ml glycerol, 40 ml H₂O, 0.45 ml 10% ammonium persulfate and 0.06 ml TEMED.

5X NFkB Binding Buffer. This 5X concentrated buffer is composed of 250 mM NaCl, 50 mM Tris Cl, 50% (v/v) glycerol, 5 mM DTT, 2.5 mM EDTA adjusted to pH 7.6. Store this buffer prior to use at -20 ° C.

[Nuclear Extract](#). Prepared from a cell line known to be positive for DNA binding protein (i.e. HeLa for NFkB).

32P-labeled DNA probe. Add 30,000-50,000 CPM double stranded consensus oligonucleotide probe per reaction mixture. For NFkB the consensus sequence is GGGGACTTTCC. As a control also prepare probe without label (cold).

Super Shift Antibody. Add recommended volume of antibody specific for DNA binding protein (usually 1 ml).

Procedure

Add the following to a microfuge tube (the volume of H₂O added should result in a total reaction volume of 20 ml including the labeled probe): poly dI-dC to 2 mg/rxn, 4 ml 5X Binding Buffer, 2.5 ml Nuclear Extract and x ml H₂O.

Gently mix the contents of the tube.

For the supershift assay add the antibody to the reaction mixture and incubate the reaction for 15 min at room temperature. Omit this step if only performing the gel shift assay.

Add the ³²P labeled probe and gently mix. Incubate the reaction for 15 min at room temperature.

Load the entire reaction mixture volume into each lane of a 5% polyacrylamide gel (1.5 mm x 20 cm x 20 cm) prepared in TGE buffer. Do not add dye to the reaction mixture lane. Dye may interfere with binding. Run the dye separately in the first and last lanes of the gel.

Run the gel at 20 milliamps for 1.5 to 2 h. Dry the gel and perform autoradiography to visualize banding patterns.

Notes

For best results let the gel polymerize for 1h then pre-run the gel for 1 h using a constant current of ~20 milliamps. Typically 2 liters of 1X TGE is used: 1.5 l in the bottom reservoir and 0.5 l in the top reservoir when using a commercially available apparatus. Do not exceed a final concentration of 100 mM sodium chloride in the reaction mixture. Concentrations above 100 mM inhibit the reaction. Do not exceed 2.5 ml of nuclear extract per reaction mixture. This is a good generalized method. Specific antibodies/probes may require altered conditions, for instance, NF-Y antibodies must be incubated for 1-2 h on ice before adding the probe. Prepare the reaction mixture in duplicate using unlabeled (cold) probe as a negative control or add cold probe and incubate 10 min at room temperature before adding labeled probe for competition experiments. Certain gel super shift antibodies are supplied with control peptides. Prepare these reaction mixtures in duplicate adding the control peptide to the reaction mixture prior to adding the antibody.

References

1. Baldwin. 1996. Ann.Rev.Immunol.14: 649-681
2. Tan, Horikoshi and Roeder. 1989. Mol. Cell Biol. 9:1733-1745.