Gel Shift Assay Protocol

The electrophoretic gel shift assay is used to detect sequence-specific DNA-binding proteins present in nuclear extracts. For NF-κB 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 as a result of binding a specific antibody to the DNA binding protein prior to reaction with the radioactive probe. 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.

I. Reagents Required

Reagent	Preparation
Molecular Biology Grade Water	N/A
Poly(dI-dC)	Use this as a non-specific inhibitor
1X Tris-Glycine-EDTA (TGE) Buffer	Prepare a 10X concentrate of 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 L. 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 H_2O, 0.45 mL 10% ammonium persulfate, and 0.06 mL TEMED.
5 NF-κB Binding Buffer	This 5X concentrated buffer is composed of 250 mM NaCl, 50 mM Tris Cl, 50% (v/v) glycerol, 5 mM DTT, and 2.5 mM EDTA adjusted to pH 7.6. Store buffer at -20°C prior to use.
Nuclear Extract	Prepared from a cell line known to be positive for DNA binding protein (i.e., HeLa for NF-κB)
32P-labeled DNA Probe	Add 30,000–50,000 CPM double stranded consensus oligonucleotide probe per reaction mixture. For NF-κB, 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).

II. Procedure

1. Add the following to a microfuge tube (the volume of H_2O 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 H_2O.
2. Gently mix the contents of the tube.
3. For the supershift assay add the antibody to the reaction mixture and incubate the reaction for 15 minutes at room temperature. Omit this step if only performing the gel shift assay.
4. Add the 32P-labeled probe and gently mix. Incubate the reaction for 15 minutes at room temperature.
5. 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 TGEbuffer. Do not add dye to the reaction mixture lane as it may interfere with binding—run it separately in the first and last lanes.
6. Run the gel at 20 milliamps for 1.5–2 hours. Dry the gel and perform autoradiography to visualize banding patterns.

III. Notes

For best results, let the gel polymerize for 1 hour then pre-run the gel for 1 hour using a constant current of ~20 milliamps. Typically, 2 L 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. Specific antibodies/probes may require altered conditions, for instance, NF-Y antibodies must be incubated for 1–2 hours 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 10minutes 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

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

Rockland products are for research use only and are not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Immunochemicals, Inc., P.O. BOX 5199, Limerick, Pennsylvania 19468, USA.

© 2018 Rockland Immunochemicals, Inc. All rights reserved.