

Technically Speaking

# Gel Shift Assay System

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*The gel shift (or electrophoretic mobility shift) assay facilitates the detection of sequence-specific DNA binding proteins. Promega's Gel Shift Assay System contains sufficient reagents for labeling target DNA and performing 100 gel shift assays including 20 controls. Here are the answers to your most common questions about the system.*

## **Q. What parameters of the Gel Shift Assay require optimization?**

We have established conditions which successfully shift several of Promega's consensus oligonucleotides with our purified transcription factors and HeLa Nuclear Extract. For novel DNA binding proteins, a variety of parameters may require optimization, including salt concentrations, detergent concentrations, incubation temperature, pH and time of the binding reaction. Metals such as zinc also may be necessary for DNA binding. If cellular or nuclear extracts are used in the assay, care must be taken to inhibit nucleases, phosphatases and proteases which could interfere with the assay. Finally, the protein:DNA complex may be unstable during electrophoresis; optimization of polyacrylamide concentration, buffer pH, ionic strength and electrophoretic temperature may enhance stability (1-3).

## **Q. What transcription factors are present in the control HeLa Extract provided with the system?**

We have successfully shifted our consensus oligonucleotides AP1, SP1, NF-kappa-B, GRE, OCT1 and CTF/NF1 in the control HeLa Nuclear Extract. One exception is AP2 consensus oligonucleotide. This is probably due to low levels of AP2 protein in the extract. As a control for AP2, we provide an *E. coli* extract containing recombinant AP2 protein.

## **Q. What carrier DNAs should I add to the binding reaction?**

If the protein of interest is a purified transcription factor, carrier DNA often is not necessary. However, if protein extracts are used, carrier DNA should be present. We recommend poly(dI-dC)·poly(dI-dC) (Pharmacia Cat.# 27-7880-01) to reduce non-specific background. Other potential carriers, such as calf thymus DNA or salmon sperm DNA, may contain binding sites for the protein of interest and interfere with the assay. The amount of carrier added to the binding reaction should be titrated carefully as the optimal range of concentration is often extremely narrow (1).

## **Q. What considerations are important in DNA probe choice?**

The size of the target DNA should be kept below 300bp to facilitate electrophoretic separation of the unbound probe and protein:DNA complex. Double-stranded synthetic oligonucleotides as well

as restriction fragments can be used as probes with the gel shift assay. Short (approximately 25bp) oligonucleotides are preferred if the protein of interest has been previously identified, since the binding site can be separated from potential binding sites for other factors. Larger restriction fragments can be utilized to map protein binding sites in putative promoter/enhancer regions. Subsequently, DNase I footprinting can be employed to determine the position of the binding site at the DNA sequence level.

**Q. How is the radiolabeled target DNA prepared?**

We include T4 Polynucleotide Kinase in the system for 5' end labeling target DNAs with [ $\gamma$ - $^{32}$ P]ATP. Alternatively, a restriction fragment probe with 5' overhangs can be labeled by filling in with the Klenow fragment of DNA Polymerase I and the appropriate [ $\alpha$ - $^{32}$ P]dNTP.

**Q. Can *in vitro* translation be used to prepare the protein of interest?**

Yes, the tRNA<sup>scd</sup>(TM) Biotinylated tRNA (cat.# L5061) has been used in conjunction with the TNT(TM) Rabbit Reticulocyte Lysate Systems to produce transcription factor AP1 (c-jun) which shifts AP1 consensus oligonucleotide (Cat.# E3201) with results comparable to recombinant AP1 (4). The TNT T7 Coupled Wheat Germ Extract System (Cat.#L4140) has been used to produce c-Rel *in vitro*. This protein specifically shifts an immunoglobulin kappa light chain enhancer probe. This interaction can be disrupted by the addition of *in vitro* translated MAD-3 (a member of the I-kappa-B family) to the c-Rel binding reaction (5). We have not tested all of our transcription factors in this manner.

**Q. How can I be sure that the shift I am observing is specific for the protein of interest?**

These methods can be utilized to verify the specificity of the protein-DNA interaction (1-3):

- The most common test of specificity is to add unlabeled target DNA to compete for the protein of interest. Specificity of binding is indicated when excess unlabeled target oligo reduces the amount of labeled protein:DNA complex, while excess non-target oligo has no effect.
- Site-specific mutagenesis of the DNA binding site can be used to examine specificity. Altering conserved nucleotides in the putative binding region may abolish protein:DNA interactions.
- Another test of specificity is the "supershift." Antibodies to the protein of interest added to the preformed protein:DNA complex can further retard its mobility (supershift) during electrophoresis.
- Antibodies to the putative DNA binding protein can be incubated with the purified protein or protein extract before incubation with labeled target DNA. The antibody may prevent protein-DNA interactions by blocking regions of the protein which bind DNA and, as a result, eliminate its ability to induce a mobility shift. Alternatively, the antibody can be used to deplete an extract of a particular protein via immunoprecipitation.

**Q. I see smearing instead of a discrete protein:DNA probe band in my gel. What may be causing this problem?**

There are several potential causes of this problem.

- Loading dyes, such as bromophenol blue or xylene cyanol, may interfere with protein:DNA interactions. Eliminate these dyes from your samples.
- Residual detergent on the glass electrophoresis plates may cause this problem. Rinse plates

- scrupulously with distilled water before pouring the gel.
- The protein:DNA complex under investigation may have a short half life and dissociate during electrophoresis. Decrease the gel running time by increasing the voltage. Lower temperature may reduce the dissociation rate of the complex. Try using chilled electrophoresis buffer or running the gel at 4°C.
  - High ionic strength buffers can destabilize protein:DNA complexes. Decrease the ionic strength of the gel and gel running buffer.

For more detailed information on troubleshooting, see references 1-3.

## References

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