

TransCruz™ Gel Supershift Assays

NOTE: Spin oligonucleotide vial before opening. Product may be lodged in vial cap.

- Label oligonucleotide probe with [³²P]-ATP to 50,000 cpm/ng by using polynucleotide kinase (for a listing of these reagents and more, see [Standard Laboratory Chemicals](#)).
- Prepare gel shift reaction buffer as follows: 10 mM **Tris** ([sc-3715](#)), pH 7.5, 50 mM **NaCl** ([sc-29108](#)), 1 mM dithiothreitol (**DTT**: [sc-29089](#)), 1 mM **EDTA** ([sc-29092](#)), 5% **glycerol** ([sc-29095](#)).
- Prepare 20 µl reaction mixture containing 3–10 µg nuclear extract and 1 µg poly dI-dC in gel shift reaction buffer. Add 0.5 ng labeled oligonucleotide probe and incubate for 20 minutes at room temperature. This constitutes the control sample for detection of DNA-protein complexes.
- To detect an antibody supershift or block of the DNA-protein complex, prepare reaction mixture as described above, also adding 1–2 µl of the appropriate TransCruz™ Gel Supershift antibody per 20 µl of reaction volume. Antibody is normally added subsequent to addition of labeled oligonucleotide probe, but result may be improved by adding antibody prior to probe. Incubate at 4° C for 1 hour to overnight, or at room temperature for 15–45 minutes.
- Resolve DNA-protein complexes by electrophoresis (25–35 ma) through a 4% polyacrylamide gel containing 50 mM Tris, pH 7.5, 0.38 M **glycine** ([sc-29096](#)) and 2 mM EDTA. Dry the gel and visualize by autoradiography.