Gel Shift Assay

Solution

1. 20 x Buffer IVT: 75 mM $MgCl_2$, 40 mM DTT, 200 mM KCl
2. Buffer E: 20 mM Hepes-NaOH (pH 7.9), 60 mM KCl, 12.5 mM $MgCl_2$, 0.1 mM EDTA, 17% Glycerol, 2 mM DTT

Protein-RNA binding reaction

1. Mix the following in a 1.5-mL tube:

Components	amount
20 x buffer IVT	1 μL
Protein (recombinant)	X μL (1 fmol ~1 pmol)
Buffer E	Y μL (X+Y=5 μL)
RNase free water	13 μL
Total	20 μL

2. Incubate at 25°C for 10 min.
3. Before RNA-protein reaction, incubate a radio-labeled RNA probe at 70°C for 30 sec, then place on ice for at least 1 min.
4. Add 1 μL of labeled RNA probe (1 fmol, 8250 cpm), then mix gently.
5. Incubate at 25°C for 15 min.
6. Add 2 μL of 80% glycerol, then mix gently.
7. Use 12 μL of reaction mixture for electrophoresis.

(Note: don't add a loading dye to the reaction mixture and separately load the dye to the gel.)

Electrophoresis and detection

1. Prepare a native polyacrylamide gel of 6% or 8% acrylamide in 1.0 x TBE or TGE buffer.
2. The gel must be pre-run for at least 20 min at 4°C, 30mA.
3. Load the samples to the gel.
4. Run at 4°C, 30 mA.
5. Run dye 2/3 of the way to the bottom of the plate.
6. After electrophoresis, remove one glass plate carefully from the gel, then dry.
7. Expose it to the imaging plate at 25°C.