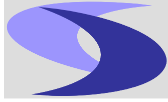

Gel Shift Assay Kit

Catalog Number GS-0001 **(For Research Use Only)**

Introduction

Electrophoretic-Mobility Shift Assay (EMSA) Kits are useful tools for identifying transcription factors that interact with their corresponding DNA consensus sequences. When a transcription factor binds specifically to a labeled dsDNA probe, and form protein/DNA complex, the complex migrates slower than non-bound dsDNA in a native (non-denaturing) polyacrylamide gel, thus resulting in shifted bands. The typical procedure is as follows: the nuclear extract is incubated with TF probe, protein/DNA complexes are separated on a non-denaturing polyacrylamide gel. The gel is transferred to a nylon membrane and detected using strepatvidin-HRP and a chemiluminescent substrate. The shiftedbands corresponding to the protein/DNA complexes are visualized relative to the unbound dsDNA. The bands are visualized after exposure to film or chemiluminescent-imaging system. The assay is non-radioactive assay with high sensitivity**.**

Materials provided with the kit

- Loading buffer (-20 °C)
- 5x binding buffer (-20 °C)
- Polyd (I-C) (-20 °C)
- 60ml Blocking buffer (RT)
- 60 µl Streptavidin-HRP conjugate (4 °C)
- 50ml 5x Detection wash buffer (RT)
- 3.6 ml Substrate A (4 °C)
- 3.6 ml Substrate B (4 °C)
- Detection sheet (RT)
- Membranes (RT)

Materials and equipment are needed

- Biotin labeled TF probe (GP-00XX, Signosis)
- 5XTBE or 10x TBE
- Gel apparatus
- Power supplies
- Stratagene UV cross-linker
- Shaker
- Imaging system or X-ray film

Assay Procedure

Forming TF-DNA complexes:

(1) Prepare nuclear extract (Nuclear extraction kit cat SK-0001 from Signosis or other nuclear extract kit or methods)

(2) Mix following components into a 0.5-ml microcentrifuge tube (PCR tube) in order

X µl Nuclear Extract (2-5 ug)
1ul poly d(I-C)
2.0 µl of 5X Binding Buffer
X µl nuclease-free water
1.0 µl of TF Probe

10ul

(3) Incubate at 16°C for 30 minutes in a PCR machine

Gel preparation

(1) Prepare 6.5% Non-Denaturing polyacrylamide Gel. Mix the following components in a 10ml centrifuge tube
1 ml of 5X TBE
2 ml of 30% Acrylamide/Bis
100 µL of 80% Glycerol
6.8 ml of deionized, sterile water
90 µL of 10% APS
10 µL Temed
Total volume is 10 mL
(2) Cast the gel as per standard protocol.
(3) Run gel in chilled 0.5X TBE for 10 min at 120V before loading samples into gel.
(4) Rinse the wells with electrode buffer (0.5x TBE) before loading samples into gel.
(5) Mix 10ul sample with 1ul loading dye
(6) Put the gel tank on ice water in ice box or run gel in a 4°C refrigerator at 100V until the dye reaches 1 inch from the bottom of the gel (Approx. time: 50-60 minutes).

Transfer

(1) Dissemble the gel cast and remove one of the plates from the gel.
(2) Transfer the gel to a glass tray filled with 0.5X TBE buffer.
(3) Soak the membrane and filter paper in 0.5X TBE.
(4) Assemble the transfer unit in the following order on the black side of cassette: one fiber pad, one piece of filter paper, gel, membrane and one piece of filter paper, one fiber pad.

(5). Make sure the gel at negative side and membrane at positive side and transfer cassette to BioRad Trans-Blot Cell and fill with pre-chilled 0.5xTBE.
(6) Transfer at 60V at for l hr in a cold room or put the tank on ice within an ice basket.
(7) After transfer, the protein-bound probe and free probe are immobilized with Stratagene UV cross-linker.

4. Detection

(1) Using forceps, transfer the membrane from the hybridization tube to a container (an empty 200 µl pipette box). Each box could have one full membrane.
(2) Rinse the membrane with 10 ml of 1X Detection wash buffer, and decant the buffer.
(3) Block the membrane by adding 15 ml of Blocking buffer for 30 minutes at room temperature with moderate shaking.
(4) Dilute 15 µl of Streptavidin-HRP conjugate with 1 ml of the 1X Blocking buffer and transfer to the container. Do not add HRP diluted solution directly onto the membrane.
(5) Continue shaking the membrane for 45 min at room temperature.
(6) Decant the Blocking buffer and wash three times at room temperature with 15 ml of 1x Detection washing buffer, 10 minutes each wash
(7) Mix equal amounts of Substrate A and B. Place the membrane on the bottom side of detection sheet on a flat surface, and overlay the membrane with 1.8 ml of substrate solution, ensuring that the substrate is evenly distributed over the membrane. Gently place the top side of detection sheet over the membrane ensure that the substrates cover the entire surface of the membrane, without trapping air bubbles on the membrane. Incubate at room temperature for 5 minutes.
(8) Remove excess substrate by gently applying pressure over the top sheet using a paper towel. Expose the membranes using either Hyperfilm (2-10 min) or a chemiluminescence imaging system (i.e., FluorChem imager from Alpha Innotech). With either method, experiment with different exposure times.

Example of gel shift assay

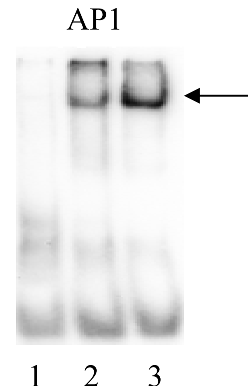

Figure: Gel shift assay analysis of AP1 DNA binding activities in HeLa and PMA-treated HeLa. 1.probe only; HeLa; 3.HeLa-PMA. The shifted bands are indicated with arrow