DATA SHEET

EMSA Assay Kit

Catalog Number GS-00XX **(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, then 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 shifted bands corresponding to the protein/DNA complexes are visualized relatively 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)
- Blocking buffer (RT)
- Streptavidin-HRP conjugate (4 °C)
- 5x Detection wash buffer (RT)
- Substrate A (4 °C)
- Substrate B (4 °C)
- Detection sheet (RT)
- Membranes (RT)
- Biotin labeled TF probe (-20 °C)

Materials and equipment are needed

- 5X TBE 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 ddH2O
9 µl

Incubate on ice for 5 minutes, then add 1.0 µl of TF Probe.

Note: For cold probe control, add 1ul of cold TF probe in the reaction.

(3) Incubate at room temperature (20-23°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
2.2 ml of 30% Acrylamide/Bis
80 µl of 80% Glycerol
6.62 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 pre-chilled 0.5X TBE buffer for 10 min at 120V before loading samples into gel.

(4) Rinse the wells with 0.5X TBE buffer 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) Soak gel, the membrane, filter paper and fiber pad in 0.5X TBE.

(4) Assemble the transfer unit in the following order on the black side (negative) of cassette: one fiber pad, one piece of filter paper, gel, membrane and one piece of filter paper, one fiber pad.

(5) Make sure to place 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) Put the membrane in a container, for example, an empty 200 µl pipette box or any container can hold 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 20 minutes at room temperature with moderate shaking. Note: Do not decant the blocking buffer, and take out 1 ml of blocking buffer for pre-dilution of Streptavidin-HRP conjugate in step 4.
(4) Dilute 15 µl of Streptavidin-HRP conjugate with 1 ml of the 1X Blocking buffer from step 3 and pour back into the container. Note: Do not add HRP diluted solution directly onto the membrane.
(5) Continue shaking the membrane for 45 min at room temperature in 15ml blocking buffer.
(6) Decant the Blocking buffer and wash three times at room temperature with 15 ml of 1x Detection washing buffer. Wash each time for 10 min with moderate shaking.
(7) Mix equal amounts of Substrate A and B for one full membrane
0.8 ml Substrate A
0.8 ml Substrate B
Place the membrane on the bottom side of detection sheet on a flat surface, and overlay the membrane with 1.6 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 to 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 solution by gently applying pressure over the top sheet using a paper towel. Expose the membranes using either Hyperfilm or chemiluminescence imaging system (i.e., FluorChem imager from Alpha Innotech). With either detection method, different exposure time could be adjusted accordingly to the images.

Example of gel shift assay

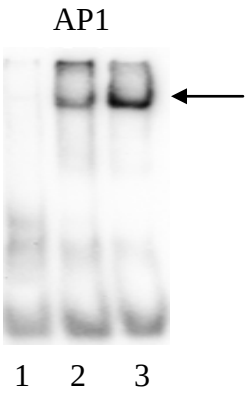

Figure: Gel shift assay analysis of AP1 DNA binding activities in HeLa and PMA-treated HeLa.
1. Probe only;
2. HeLa;
3. HeLa-PMA;
The shifted bands are indicated with arrow.