Signosis, Inc.
Innovative Plate Assay Solutions

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 (TF) that interact with their corresponding DNA consensus sequences. When a TF binds specifically to a labeled dsDNA probe and forms a protein/DNA complex, the complex migrates slower than non-bound dsDNA in a non-denaturing polyacrylamide gel, thus resulting in shifted bands.

Procedure

The nuclear extract is incubated with TF probe, and then protein/DNA complexes are separated on a non-denaturing polyacrylamide gel. The gel is transferred to a nylon membrane and detected using Streptavidin-HRP Conjugate and a chemiluminescent substrate. The shifted bands corresponding to the protein/DNA complexes are visualized in comparison 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 EMSA Kit

Component	Qty	Store at
NB Membrane	2	RT
Detection Sheet	2	RT
5X Detection Wash Buffer	50 mL	RT
Streptavidin-HRP Conjugate	60 µL	4°C
Substrate A	1.2 mL	4°C
Substrate B	1.2 mL	4°C
Blocking Buffer	60 mL	RT
5X Binding Buffer	60 µL	-20°C
Polyd(I-C)	30 µL	-20°C
10X Loading Buffer	50 µL	-20°C
TF Probe (Hot)	30 µL	-20°C
Cold TF Probe	30 µL	-20°C

Required items but not provided:

- Signosis, Inc. Nuclear Extraction Kit – Cat# SK-0001
- 2 liters of 0.5X TBE (dilute from 10X or 5X)
- 30% Acrylamide/Bis, 80% Glycerol, 10% APS, and TEMED (see volume requirements on Page 2)
- Electrophoresis equipment and related components
- Stratagene UV Cross-Linker
- Plate-shaker
- Imaging system or X-ray film
- PCR Machine and 0.5 mL PCR tubes
- Laboratory ice bucket

Please Read Assay Procedure Before You Begin

Assay Procedure

Forming TF-DNA complexes:

1. Prepare nuclear extract.
   a. **Signosis, Inc.** *Nuclear Extraction Kit* (Catalog # SK-0001) is recommended to acquire your nuclear extract for the EMSA assay.
2. Obtain a laboratory ice bucket, and fill with ice.
   a. Take the following components below in Step (3) and place on ice.
3. Once all components are thawed, centrifuge briefly to bring all contents to bottom of each component tube. Mix following components into a 0.5 ml PCR (or microcentrifuge) tube in order:

X µl	Nuclear Extract (2-5 µg)
1 µl	Poly D (I-C)
2 µl	5X Binding Buffer
Y µl	Nuclease-Free ddH2O
9 µl	

   Incubate on ice for 5 minutes, then add 1.0 µl of TF Probe (Hot).
   ** **Note:** For cold probe competition control, add 1ul of cold TF probe in the reaction. **

   X – Volume of Nuclear Extract within range of 2-5 µg. **As a control, add Nuclease Free ddH_2O instead of Nuclear Extract.
   Y – volume of Nuclease Free ddH_2O to bring the total volume up to 9 µL. If the volume is already 9 µL, this component does not need to be added.

4. Incubate the tubes at **22ºC** for **30 minutes** in a PCR machine.

Gel preparation

5. Prepare a **0.5X** *TBE Buffer Solution*, and place on ice or refrigerator for at least **30 minutes.**
6. Prepare 6.5% non-denaturing polyacrylamide gel. Mix the following components in a 10 mL tube:

Component	Volume
Deionized, sterile H_2O	6.62 mL
5X TBE	1 mL
30% Acrylamide/Bis	2.2 mL
80% Glycerol	80 µL
10% APS	90 µL
TEMED	10 µL

7. Immediately transfer mixture into a gel cast and place the gel comb on top of the gel as per standard protocol. The gel should take **20 to 30 minute**s to solidify.
8. Once the gel is solidified, place the gel in the *gel electrophoresis apparatus*. Fill the gel-dam completely with pre-chilled *0.5X TBE Buffer*. Fill the tank to the 2-gel line with 0.5X *TBE Buffer as well*. Run the gel for **10 minutes** at **120V**.
9. Turn off the apparatus that is applying current to the gel. With the apparatus lid removed, expunge any residual gel-mixture content in the wells that failed to polymerize with *0.5X TBE Buffer*. You can use a 200 µL pipette to accomplish this.
10. Mix **10µL** *sample* from Step (4) with **1µL** *10X Loading Buffer*. Add one sample per well and place the gel tank's lid back on.
11. Surround the gel tank with ice in a bucket so that the ice is packed to the bottom of the lid. You can also run gel in a 4°C refrigerator. Run the gel at **100V** until the dye reaches **1 inch** (2.54cm) from the bottom of the gel, which takes about **50** to **60 minutes**.

Transfer

12. Pour **300 mL** of *0.5X TBE Buffer* into a 6x6x2 inch (15x15x5cm) container. Place the transfer cartridge so that the black plastic (negative) side is submerged in the buffer. Place **1 filter sponge** on top of the black side, followed by **1 piece of filter paper**.
13. Turn off the power of the gel electrophoresis unit and dissemble the gel cast by removing one of the plates from the gel cast. Be gentle with the removal of the gel or it will tear.
14. After successfully removing the gel, place and align the gel on the filter paper from **Step (12).**
15. Acquire a **NB Membrane** provided by the kit. Make an incision on the top-right corner of the membrane to help orient you for the **Detection phase**.
16. Assemble the remainder of the transfer unit by briefly soaking the *NB membrane* in *0.5X TBE Buffer* and placing it on top of the gel. Briefly soak the 2nd filter paper and filter sponge. Place 2nd filter paper on top of the *NB membrane* and the filter sponge on top of the filter paper. Close the transfer cassette.
17. Place the cassette into the *blotting apparatus.* Properly orient the cassette so that the gel contents will run in the direction of the membrane (towards the anode). Fill the rest of the gel apparatus so that the cassette is completely submerged with pre-chilled *0.5X TBE Buffer*.
18. Place the lid on the *blotting apparatus*. Run the transfer at **60V** for **l hour** in a cold room or put the tank surrounded by ice inside an ice basket.
19. After the hour has elapsed, extract the *NB Membrane* from the cassette and place the membrane in a clean plastic container of equal size to the membrane (such as a 200 µL pipette tip box).
20. Immobilize the protein-bound probe and free probe using the **Stratagene UV Cross-linker**. If you do not have this device, exposing the membrane to UV light at **120,000 µjoules** for **1 minut**e will suffice.

Detection

21. Put the *NB Membrane* in a container, for example, an empty 200 µL pipette box or any container can hold one full membrane.
22. Rinse the *NB Membrane* with **10 mL** of *1X Detection Wash Buffer* and decant the buffer.

23. Block the *NB Membrane* by adding **15 mL** of *Blocking Buffer* for **20 minutes** at room temperature with moderate shaking on the *Plate-shaker*. After the 20 minutes have elapsed, take out **1 mL** of *Blocking Buffer* that is currently washing over the *NB Membrane* to use for diluting the Streptavidin-*HRP Conjugate* in Step (24).
24. Dilute **15 µL** of *Streptavidin-HRP Conjugate* with **1 mL** of the *1X Blocking Buffer* from Step (23) and pour back into the container. Note: Do not add HRP diluted solution directly onto the membrane.
25. Continue shaking the membrane for **45 minutes** in the *Streptavidin-HRP Conjugate/Blocking Buffer* solution.
26. Decant the *Blocking Buffer* and wash the membrane three times at room temperature with **15 mL** of *1X Detection Wash Buffer*. Wash each time for **10 minutes** with moderate shaking on the *Plate-shaker*.
27. Mix equal amounts of **0.55 mL** *Substrate A* and *B* for one full membrane, totaling **1.1 mL** *Substrate Solution*. Place the *NB Membrane* in between the flaps of the provided *Detection Sheet*.
28. Place the membrane on the bottom flap of *Detection Sheet* on a flat surface and cover the *NB Membrane* with **1.1 mL** *Substrate Solution* using a 1 mL pipette. Once the substrate is evenly distributed over the membrane, gently place the top flap of *Detection Sheet* over the *NB Membrane* to ensure that the *Substrate Solution* covers the entire surface of the *NB Membrane*, without trapping air bubbles on the membrane. Incubate at room temperature for **5 minutes.**
29. 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 Image of AP1 Experiment:

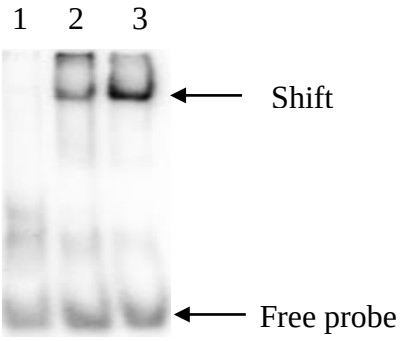

Figure 1: Gel shift assay analysis of AP1 DNA binding activities in HeLa and PMA-treated HeLa.
1. Probe only;
2. HeLa;
3. HeLa-PMA;
The shifted bands are indicated with arrow.

Figure 2: Assay Overview

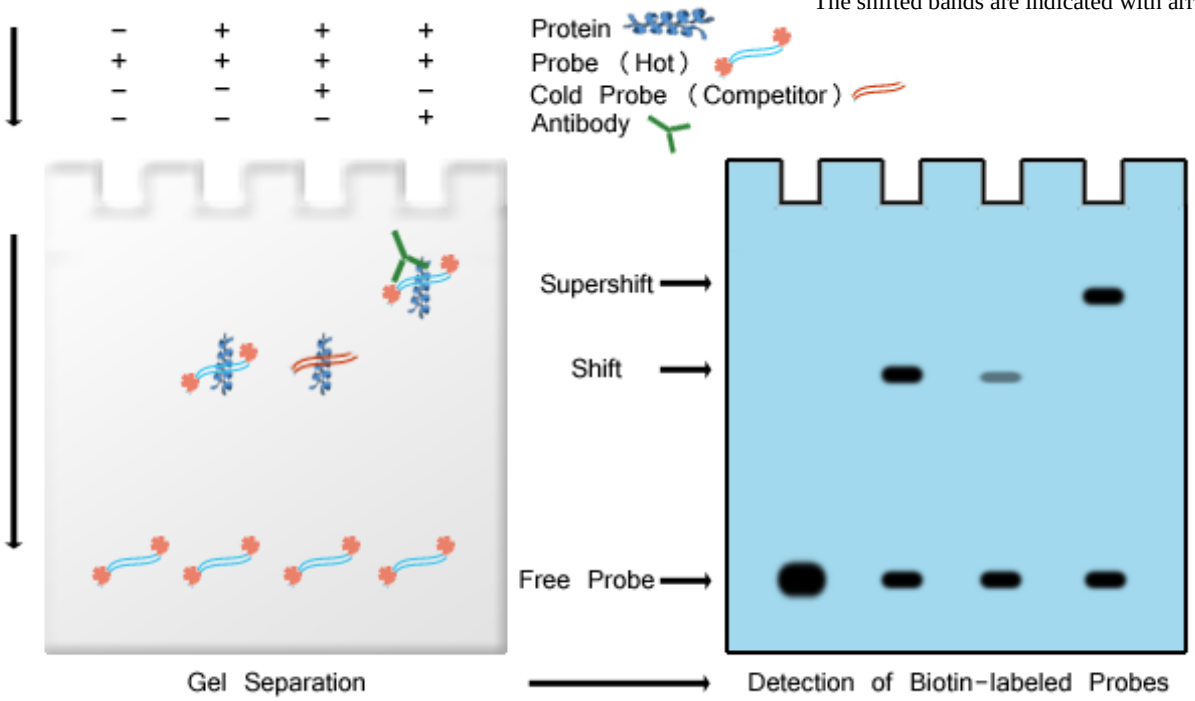

Figure 2 – the Cold Probe functions as a competitor, resulting in a band that is smaller and incredibly faint. This kit **does not** come with a provided antibody for this assay. The *Supershift* is shown as a reference for specific protein-DNA binding interaction.