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 oC)
- 5x binding buffer (-20 oC)
- Polyd (I-C) (-20 oC)
- ☐ Blocking buffer (RT)
- ☐ Streptavidin-HRP conjugate (4 oC)
- ☐ 5x Detection wash buffer (RT)
- ☐ Substrate A (4 oC)
- ☐ Substrate B (4 oC)
- ☐ Detection sheet (RT)
- ☐ Membranes (RT)
- ☐ Biotin labeled TF probe (-20 oC)
- ☐ Cold TF probe (-20 oC)

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- Cold TF probe (-20 oC)

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$_o$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$_o$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.