GENTAUR

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 Coading buffer (-20 oC) Polyd (I-C) (-20 oC) Blocking buffer (RT) Streptavidin-HRP conjugate (4 oC) Sx Detection wash buffer (RT) Substrate A (4 oC) Detection sheet (RT) Membranes (RT) Blotin labeled TF probe (-20 oC) Cold TF probe (-20 oC) Sx binding buffer (-20 oC) Sx binding buffer (-20 oC) Blocking buffer (-20 oC) Sx binding buffer (-20 oC) Streptavidin-HRP conjugate (4 oC) Sx Detection wash buffer (RT) Substrate A (4 oC) Sx binding buffer (-20 oC) Sx binding buffer (-20 oC) Blocking buffer (RT) Substrate A (4 oC) Detection sheet (RT) Biotin labeled TF probe (-20 oC) Cold TF probe (-20 oC) Materials and equipment are needed SX TBE or 10X TBE Gel apparatus Power supplies Stratagene UV cross-linker
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Assay Procedure
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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_oC) 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 ul 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_oC 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.