DATA SHEET

EMSA "Gel Shift" Kit

Introduction to EMSA Kits
Shift Assay (EMSA) Kits are useful tools for identifying proteins that interact with DNA. This rapid technique is based on the separation of free DNA from protein/DNA complexes due to the differences in their electrophoretic mobility in native (non-denaturing) polyacrylamide gels. When a protein binds specifically to a labeled dsDNA sequence, it migrates slower than non-bound dsDNA in a polyacrylamide gel, thus resulting in discrete bands corresponding to the individual protein/DNA complex. A typical EMSA experiment is performed by incubating a biotin-labeled transcription factor (TF) Probe with treated and untreated nuclear extracts. The 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 relative to the unbound dsDNA. The bands are visualized after exposure to film or chemiluminescent-imaging system (Figure 1).
For more specific binding, you may wish to add unlabeled specific dsDNA probe (cold probe; provided) to the protein/DNA reaction mixture which competes with the labeled dsDNA probe (biotin-labeled probe) for binding to the protein. This causes the labeled probe to migrate to the bottom of the gel and reduces the intensity of the shifted band.The EMSA Kits are suitable for validation of results obtained using Procarta® Transcription Factor Assay, Protein/DNA Arrays (Cat. #s MA1010 to MA1015) or to validate binding activity of a specific transcription factor (protein) to DNA. Enough reagents are provided to perform 25 binding reactions. TF EMSA Kits measure the activity of specific transcription factors in nuclear extracts. The assay is highly specific, precise, and requires 4-8 µg of protein/well. We currently offer more than 400 EMSA assays.

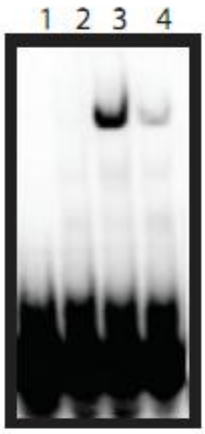


Figure 1: Example EMSA image. Lane 1: Labeled EMSA Probe only with no sample, Lane 2: Labeled EMSA Probe with untreated sample. Lane 3: Labeled EMSA Probe with treated sample. Lane 4: Treated sample with cold and labeled EMSA probes.

Kit Contents and Storage
The EMSA Kit contains the following components. Refer to the product insert for quantities and details of components supplied. If stored properly, reagents have a shelf-life of 6 months.
Box 1 is shipped on dry ice. Please store at -20°C upon receipt

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

Box 1 Components	Description	Volume	Storage
5X Binding Buffer	Aqueous buffered solution for TF binding	50 µL	–20°C
Poly d(I-C)	Used in TF binding reactions to act as competitor to TF Probe	25 µL	–20°C
Loading Dye	Dye for loading samples and monitoring electrophoresis and transfer	60 µL	–20°C
2X Blocking Buffer	Aqueous buffer for blocking during detection	50 mL	–20°C
Control Nuclear Extract	Nuclear Extract prepared from HeLa cell line for use with control probe and cold control probe	5 µL	–20°C
Control Probe	Control reagent	10 µL	–20°C
Cold (unlabeled) Control Probe	Control reagent	10 µL	–20°C
Distilled H_20	Distilled, purified water	500 µL	–20°C

Box 2 is shipped on blue ice. Please store at -4°C upon receipt.

Box 2 Components	Description	Volume	Storage
Solution I	Chemiluminescent detection reagent (1 mL)	1 mL	4°C
Solution II	Chemiluminescent detection reagent (1 mL)	1 mL	4°C
Solution III	Chemiluminescent detection reagent (8 mL)	8 mL	4°C
Streptavdin-HRP Conjugate	Used for detection of biotinylaled oligo on membrane (100 µL)	100 µL	4°C
10X Detection Buffer	Aqueous buffer for detection step (10 mL)	10 mL	4°C
10X Wash Buffer	Aqueous buffer for washing after addition of streptavidin-HRP (30 mL)	30 mL	4°C

Plastic Pouch is shipped on dry ice. Please store at -20°C upon receipt.

Plastic Pouch	Description	Volume	Storage
TF Probe	TF-specific Probe, biotinylated	1 vial	–20°C
Cold (unlabeled) TF Probe	TF-specific Probe for use in competition assay	1 vial	–20°C

Required Equipment and Materials Not Provided

Reagents

Item	Source
30% Acrylamide/Bis Solution	Bio-Rad P/N 161-0156
Glycerol	Sigma P/N G5516
Ammonium Persulfate (APS)	Bio-Rad P/N 161-0700
TEMED	Bio-Rad P/N 161-0800
10X TBE Stock Solution (1.0 M Tris, 0.9M Boric Acid 0.01 M EDTA)	Invitrogen P/N 15581-044
Nuclear Extraction Kit	Panomics P/N AY2002
DC Protein Assay Kit, Protein Determination Kit	Bio-Rad P/N 500-0122

Equipment

Item	Source
Electrophoresis Unit	Mini-PROTEAN® II (P/N 165-2944 from Bio-Rad)
Electroblotting Device	Mini Trans-Blot® (P/N 170-3930 from Bio-Rad)
Dry Oven	Major Lab Supplier
UV Cross Linker (optional)	Stratalinker® (P/N 400071 from Stratagene)
Chemiluminescent Imaging System (optional if using X-Ray film)	FluorChem® From Alpha Innotech
Rotating shaker	Major Lab Supplier
Thermal cycler	Major Lab Supplier

Materials

Item	Source
Blotting Paper	Whatmann® 3MM Paper
X-Ray Film (optional if using Chemiluminescent Imaging system)	Hyperfilm™ ECL™ (P/N RPN3114K from GE Healthcare)
Positively Charged Nylon Membrane, 0.45 µm	Biodyne® B, (P/N 60201 from Pall)

Guidelines for Assay Design and Analysis
Preparing Samples The protein concentration of the nuclear extract inputs should be at least 1 µg/µL. If the samples can not reach this minimum concentration, you may need to prepare new nuclear extracts. You can either increase the number of cells used during the process or use less Buffer B when the pellet is resuspended during the final incubation step of the nuclear extraction process.
General Guidelines ♦ Read this user manual and all product inserts before performing the assay.
♦ Store all reagents at the recommended temperatures.
♦ Use Nuclear Extraction Kit for best results.
Assay Procedures
Before You Start ♦ Thaw Positive Control and sample nuclear extracts on ice.
♦ Thaw TF-Specific Probe and TF-Specific Cold Probe on ice.
Preparing Nuclear Extracts
We recommend using a commercially available kit, such as Nuclear Extraction Kit (P/N AY2002). Please note that the provided control nuclear extract is a positive control only when used with the supplied control probe.
♦ We recommend a minimum of 4 µg of nuclear extract per lane and loading multiple wells with varying concentrations to determine the optimal nuclear extract concentration.
♦ You will need adjust the amount of nuclease free water accordingly so that the

total volume of nuclear extract and nuclease free water is no more than 6 µL.
♦ When measuring protein concentration, we only recommend using the DC Protein assay from Bio-Rad (Lowry assay) when used in conjunction with our Nuclear Extraction Kit.

Forming TF-DNA Complexes

Forming TF-DNA complexes:

Step	Action
1	Prepare nuclear extract
2	For each nuclear extract sample, combine the following components into a sterile 0.5-mL microcentrifuge tube (PCR tube). • 2.0 µL Nuclear Extract (at a concentration of 2 µg/µL) • 1.0 µL Poly d(I-C) (1 µg/µL) • 2.0 µL of 5X Binding Buffer • 4.0 µL nuclease-free water Mix above reagents and incubate at RT for 5 minutes
3	Add 1.0 µL of TF Probe and proceed to step 6. Total volume should be 10 µL.

Forming TF-DNA complexes: *(continued)*

Step	Action
4	(OPTIONAL) If a competition assay is desired (see Figure1, Lane 4), follow steps 4 and 5. • 2.0 µL Nuclear Extract (at a concentration of 2 µg/µL) • 1.0 µL Poly d(I-C) (1µg/µL) • 2.0 µL of 5X Binding Buffer • 2.0 µL nuclease-free water Mix and incubate 5 min at room temp
5	Add 2.0 µL Cold TF Probe to the above mixture and incubate for 5 minutes at RT. Then add 1.0 µL of labeled TF Probe and proceed to Step 6.
6	Incubate samples at 15°C for 30 min in a thermal cycler

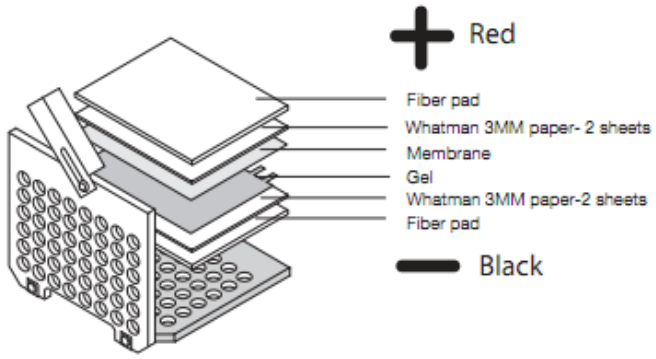
Gel Preparation

Non-Denaturing Gel

Step	Action
1	Prepare and cast a 6.0% non denaturing polyacrylamide gel. Be sure to dilute solutions/buffers, as described in the Appendix. Mix the following components into a sterile 50-mL centrifuge tube (add in order listed): • 1 mL of 10X TBE • 4 mL of 30% Acrylamide/Bis • 625 µL of 80% Glycerol • 14.375 mL of deionized, sterile water • 300 µL of 10% APS • 20 µL TEMED Total volume is 20 mL
2	Pre-chill 0.5X TBE to 4°C before running your gel.
3	Run gel in chilled 0.5X TBE for 10 min at 120V before loading samples into gel. Prior to loading your samples, flush the wells with a transfer pipet.
4	Mix samples with 1 µL of Loading Dye provided and load 10 µL of sample to each lane.
5	Run the gel at 4°C (in an ice bath or refrigerator) at 120V until the dye reaches 1 inch from the bottom of the gel (Approx. time: 50-55 min).

Transfer

Step	Action
1	Presoak Pall Biodyne B nylon membrane in 0.5X TBE. Prepare four sheets of gel-sized Whatman 3MM paper (8 x 10 cm). Presoak two sheets of Whatman 3MM paper in 0.5X TBE. **IMPORTANT** Please ensure that only Pall Biodyne B nylon membrane is used. Any other membrane may cause high backgrounds
2	After electrophoresis, carefully remove one glass from the gel. Cover with one sheet of dry Whatman 3MM paper and the gel will stick to the paper. Gently lift the Whatman paper and gel away from the glass plate and note the orientation of the gel. Add an additional Whatman paper and soak in 0.5X TBE. Sandwich the gel with the pre-soaked Biodyne B membrane and two sheets of presoaked Whatman paper then add the fiber pads on both ends. See illustration below

Step	Action
3	Place the sandwich in an electoblotting device and fill tank with 0.5X TBE. Transfer for 30 minutes at 300mA. **IMPORTANT** Ensure that the sandwich is oriented properly. The Biodyne B membrane should be closest to the positive pole (red) and the polyacrylamide gel should be closest to the negative pole (black). See Figure 2 below.
	Figure 2 

Immobilization and Detection

- **100 mL or 1X Blocking Buffer:** To 50 mL of deionized water, add 50 mL of 2X Blocking Buffer provided. Mix well and store at 4°C
- **300mL of 1X Wash Buffer:** To 270 mL of deionized water, add 30 mL of 10X Wash Buffer provided. Mix Well and store at room temperature
- **100mL of 1X Detection Buffer:** To 90 mL of deionized water, add 10 mL of 10X Detection Buffer (provided). Mix well and store at room temperature.

Step	Action
1	After transfer, remove the membrane from the sandwich of Whatman paper and gel and place between two fresh sheets of Whatman 3MM paper. **IMPORTANT** If transfer was successful, the loading dye should be slightly visible on the membrane.
2	The oligos on the membrane can be fixed by baking the membrane for 1 hour in a dry oven at 80°C. Alternatively, the oligos can be fixed using a UV crosslinker for 3 min. If UV crosslinking, ensure that the side of the membrane that was closest to the gel is exposed to the UV light source. **Note** At this point in the experiment, you can choose to continue or stop at this point. The membrane is stable for several months if stored between Whatman paper and in the dark.
3	Transfer the membrane to a new container containing 20 mL of 1X Blocking Buffer. If more than 1 membrane was prepared, each membrane will need its own container. Lids from a 200 µL pipette tip box can be used for 8 x 10 cm blots.
4	Block the membrane by incubating at room temperature with the 1X Blocking Buffer for 15 min with gentle shaking.

Step	Action
5	Remove 1 mL of the 1X Blocking Buffer from the blot container and place into a clean microcentrifuge tube. Add 20 µL of Streptavidin-HRP to the tube and vortex for 10 seconds.
6	Transfer the diluted Streptavidin-HRP mixture to the container with the blot and continue shaking at room temperature for another 15 min. **IMPORTANT** When adding the mixture to the container, avoid pouring the contents of the microcentrifuge tube directly onto the membrane
7	Decant the diluted Steptavidin-HRP solution. Wash each membrane for 8 minutes, 3 times at room temperature with 20 mL of 1X Wash Buffer.
8	Decant any excess wash buffer and then add 20 mL of 1X Detection Buffer to each membrane and incubate at room temperature for 5 min.
9	Take a plastic sheet protector and cut the sheet protector so that the membrane fits between the inside of the pocket of the two sheets. Alternatively two pieces of transparency film can be cut and the membrane sandwiched between each piece.
10	Prepare 2 mL of working Substrate Solution for each membrane by mixing (in order): 200 µL Solution I with 200 µL Solution II, briefly vortex, then add 1.6 mL of Solution III and mix.
11	On a flat and even surface, remove the top plastic sheet of the "sandwiched" membrane and pipette 2 mL of the mixed working Substrate Solution onto each membrane. Replace the top plastic sheet and ensure that the substrate solution is evenly distributed over the membrane with no air bubbles. Incubate at room temperature for 5 min. **IMPORTANT** Remove excess substrate by gently applying pressure over the top sheet and using a paper towel to wipe up any excess fluid.
12	Expose the membranes using either Hyperfilm ECL (2-10 minutes) or a chemiluminescent imaging system (12-15 min), such as the FluorChem Imager from Alpha Innotech Corp. **IMPORTANT** Several different exposure times to film or the imaging system may be needed for an optimal EMSA image.

Troubleshooting

Possible Problems and Recommended Solutions

Observation	Possible Cause	Recommended Action
Weak or no signal on free probe	Insufficient Transfer	Follow instructions in the user manual for performing the assay.
	Exposure Time to Short	Increase time of exposure
	Membrane is too dry	Keep membrane moist during detection
High background	Samples overdeveloped	Shorten the development time.
	Non-specific protein bound to membrane	Increase blocking or washing times.
	Wrong type of membrane used	Only use Biodyne B, a positively charged nylon membrane.
No Shift Observed	Not enough protein used	The amount of extract will vary according to the extract preparation, DNA binding affinity of the protein, and quality of the extract. We recommend using a minimum of 4 µg of nuclear extract protein should give a sufficient band shift result
	Insufficient protein	Confirm the concentration of the protein assay using a Lowry assay. If needed, increase the amount of protein in the nuclear extract. We recommend preparing nuclear extracts with Panomics' Nuclear Extraction Kit (P/N AY2002).
	Target protein not activated (induced)	Review induction procedures. You may need to change cell lines, inducer, or induction conditions.
There is no clear band in the lane	Disassociation of protein and probe has occurred during running of the gel	Keep all buffers and other components chilled while running the gel to minimize disassociation
There are multiple bands in the lane	Some non-specific binding of probes can occur	Determine specific bands by performing competition reactions with supplied unlabeled probes.