Product Information

G-Fast® non-radioactive electrophoretic mobility shift assay (EMSA) Kit

Catalog Number: G-EMSA-01 (20 Assays) **Stored at 2-8°C**

Description:

This kit provides an innovative technology and a fast, simple procedure to study the protein:nucleic acid (DNA/RNA/oligo) interactions (DNA/RNA-binding activities in a target protein) without using radioisotope, biotin, digoxygenin or chemiluminescent substrate reagents for EMSA "**super-shift**" visualization in a native polyacrylamide or agarose gel.

- No prelabel DNA/RNA/protein procedure (e.g. a radioisotope, digoxygenin, biotin or a fluorescent dye) before the binding reaction;
- No interference with the formation of the nucleic aid-protein complex during the binding reactions.
- No gel fix and destain, no chemiluminescent substrate reagents are required for EMSA visualization.

Kit Contains:

Components	Benefits and Features
EMSA DNA precipitation solution (2x) (Cat.: PS-01D) Size: 1.0ml	• Rapid purification and concentration of DNA fragments (more than 100bp sizes) from PCR amplified products, raw DNA extractions or digestion products. • Effectively remove the unnecessary primers-dimers, dNTPs, fluorescence dyes, free oligonucleotides and the enzymes, which may interfere the EMSA binding reaction.
EMSA nuclear protein extraction kit (Cat.: NP-02) Size: 20 extractions	• Designed for rapidly extracting intact, non-denatured nuclear protein fragments from various cell types or tissues without freeze/thaw cycles or sonication, prepared for EMSA .
EMSA DNA/RNA loading buffer (6x) (Cat.: LB-002) Size: 200 ul	• Stain nucleic acids (DNA/RNA) during electrophoresis. • An environmentally friendly DNA/RNA staining dye, view DNA on gels with blue light or UV light. • Sensitivity: down to 1-5ng dsDNA under UV light.
Fast protein in-gel staining solution (Cat.: PS-001) Size: 2.0 ml	• Ultra-fast protein staining - no fix and destain steps are required. Obtain results within 15 minutes or less.
Quick protein gel drying solution (Cat.: NP-02) Size: 60 ml	• Air-Dry the polyacrylamide gel evenly without cracking. Only 15 minute incubation before air drying. Non-toxic - ideal for fluorography, densitometry, autoradiography, or permanent gel storage .
10x EMSA binding buffer size: 100ul 10x EMSA annealing buffer size: 100ul	• Ready-to-use optimized buffers for annealing and binding reactions.

Protocol: (Keep all buffers and Nucleic acid/Protein samples on ice)

1. **Prepare Nucleic acids (DNA/RNA/Oligo probes):**

- *Probe length and concentration: 20bp-5.0kbp and 20ng-1.2ug per reaction.*
- *Short probes (20bp~80bp) may be directly from commercially synthesized, annealed and desalted.*
- *Longer probes ((80~5.0k bp) may be prepared by PCR-amplified or restriction digestion.*
  ***Note:** *gel- or HPLC-purification may be required.*

(1) Protocol to produce a 20bp-80 bp double-stranded probe from the **complementary oligos or single stranded DNA/RNA.**

- Design and prepare the complementary oligonucleotides/ssDNA/RNA (~300-800ng).
  Note: **Calculate the DNA concentration with formula: OD260 x dilution fold x 33 / 1000 = (ug/ul)

- Resuspend the two purified oligos in 9 µl dH_2O with 1 µl 10X Annealing buffer.
- Mix equal amount of the complementary oligonucleotides/ssDNA/RNA in a clean tube and place in a 50°C- 95°C (See n**ote:** ***) water bath or a heating block for 2-5 minutes, then cool down slowly at room temperature for 10-15 minutes.
- Keep the annealed DNA on ice for the binding reactions.

Note: *** *The temperature may vary for annealing interms of your DNA sequence.*
Note: **** *Calculate the annealed DNA concentration with formula: OD260 x dilution fold x 50 / 1000 = (ug/ul)*

(2) Protocol to purify the **PCR amplified fragments or digestion products** (80bp~5.0k bp) with DNA Precipitation Solution (Cat. PS-01D) to remove the unnecessary primers-dimers, dNTPs, fluorescence dyes, free oligonucleotides, the enzymes used to generate the fragments.

Note: Alternative DNA purification with Zmtech agarose gel DNA extraction kit (cat.:GD-01D) or QIAGEN PCR spin column kit;

1. Add an equal volume of DNA Precipitation Solution (2x) into the PCR products or digestion products. Mix well and incubate at room temperature for 5 minutes.
2. Centrifuge at 12,500 x g for 10 minutes at 4°C.
3. Carefully aspirate liquids and simply rinse tubes with 2 volumes of 80% ethanol for 2 times without resuspending the DNA pellets. The DNA pellets will **not** be visible if the concentration is less than 20ng/ul.
4. Dissolve the DNA pellets in 10-30µl of **nuclease-free** TE buffer or distilled water if the DNA pellet is visible. Otherwise, use 5ul of **nuclease-free** TE buffer or distilled water.
5. Measure DNA concentration using a spectrometer and store DNA at -20°C.

2. **Prepare Proteins** (poly-peptides, antibodies, purified proteins or Nuclear extraction proteins):

- *Protein concentration and source: Optimize the target protein concentration per reaction in terms of the protein:DNA molar ratio (see the steps of **setup the binding reactions**).*
The source of proteins may be a crude nuclear or whole cell extract, synthesized/ transcription product or a purified protein.

Procedure for preparing the nuclear protein extracts from cells/tissues:

1. Add 250ul ice-cold cytoplasmic lysis buffer into a clean 1.5ml microcentrifuge tube containing 25mg cells (approximately 25ul packed cells or 1x 10e6 cells) or 40mg frozen/ fresh tissues.
2. Gently pipette up and down several times and incubate on ice for 10 minutes.

Note 1. For preparation of cells, harvest cells from culturing wells/plates/flasks as usual and wash cells once with 1x ice-cold PBS/DPBS, centrifuge at 1,600 rpm for 8 minutes, aspirate liquids.
For tissue preparation, chop tissues into small pieces using a clean razor blade. This step may help to completely lysate cells/tissues.
Note 2. The buffer volumes used in all steps need to coincide with the weight of tissue/cell preparation.

3. Homogenization (**0ptional**): using a pre-chilled, clean Dounce homogenizer to homogenize the tissues twice at speed 4 (moderate) speed for 20 seconds.
Or using the pre-chilled Teflon pestle homogenizer to homogenize the tissues/cells 15-20 strokes on ice.
Or using a syringe with a needle gauges between 25 and 28. Pass cells/tissues through needle about 30-50 times to disrupt the cell membrane and release the intact nucleic.
4. Add 15ul of detergents, vortex vigorously at highest speed for 10 seconds. Centrifuge at 12,000 xg for 30 seconds at 4°C. Aspirate liquids.
5. Add 200ul cytoplasmic washing buffer to resuspend the pellet. Centrifuge at 12,000 xg for 30 seconds at 4°C. Aspirate liquids. (The remained cytoplasmic fractions were washed out).
6. Resuspend the pellet in 50ul nuclear lysis buffer and vortex vigorously for 10 seconds.

7. Incubate suspension for 30 minutes on ice (vortex 10 seconds every 10 minutes).
8. Centrifuge at 12000xg for 10 minutes at 4°C. Transfer the supernatant (**the nuclear protein fractions**) to a clean prechilled microcentrifuge tube.
9. Determine the nuclear protein concentration by Bradford or BCA Assay. Store all the extracts aliquots at -80°C.

3. **Setup EMSA binding reactions:**

Set up the EMSA binding reactions (total volume:20ul) & optimize the ratio of protein/DNA:

Exp.#:	*Protein concentration (mg/mL)*	*DNA concentration (ug/ul),*	*10x EMSA binding buffer,*	*water*
1.	0 ul	1 ul	2 ul	17ul
2.	1 ul	1 ul	2 ul	16ul
3.	2 ul	1 ul	2 ul	15ul
4.	4 ul	1 ul	2 ul	13ul
5.	6 ul	1 ul	2 ul	11ul
6.	8 ul	1 ul	2 ul	9 ul
7.	10 ul	1 ul	2 ul	7 ul
8.	15 ul	1 ul	2 ul	2 ul

4. **EMSA reaction incubation and non-denaturing gel electrophoresis & "super-shift" detection:**

1. **Immediately transfer the reaction tubes to a water bath or thermocycler set to 55-70°C and incubate for 10-30 minutes.**

   Note: The temperature and time may vary for your protein. Start with 55°C for 20 minutes if you are unsure.

   Note: continue the step 3 if not do the binding reaction with target antibodies.

2. (**Optional**) Add 1ul target antibody (1mg/ml) into the EMSA reaction tube and incubate at room temperature or 37°C for 45 minutes.
3. Prepare the 4-6% native polyacrylamid gel during the incubation time and Pre-Run Gel for 10-20 minutes at 100V for an 8 × 8 × 0.1cm gel before loading the reaction sample.
4. After the reaction incubation, Add 4μL of 6X EMSA DNA/RNA Loading Buffer (Cat: LB-002) to each 20μL binding reaction, pipetting up and down several times to mix. DO NOT vortex or mix vigorously.
5. Load samples directly into the gel wells and run the gel at 200 V for 20-30 minutes (or more), depending on the length of the DNA probe.
6. View DNA bands using a blue light transilluminator during or after electrophoresis.
7. Images can be taken using a blue light transilluminator or a UV transilluminator.

5. **Protein stain and gel dry** (Optional):

Microwave In-gel Protein Staining Protocol:

- After DNA images, place the mini-gel directly in a plastic/glass container contained ~50 ml H_2O and 500ul of protein in-gel staining solution.
- Gentle mix well and microwave for 20-30 seconds or until the solution almost boils.
- Gentle shake the gel on an orbital shaker for 10 minutes and discard the staining solution.
- Wash the stained gel once with deionized water for 5 minutes on a rotary shaker.
- Photograph the stained gel or continue gel dry steps for long term storage.

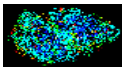
Polyacrylamide Gel Drying Protocol:

- Place the gel directly into a clean petri dish (10cm²) or a container fitted the gel size.
- Add enough Quick protein gel drying solution to cover the gel.
  Note: For 8 x 10 cm mini- gel use approximately 25ml-30ml of Gel - Drying Solution.
- Shaking gently on a shaker for 5-10 minutes.
  Note 1: Gel might shrink uniformly (without cracking) by a factor of about 30-50%. **Note 2:** Do not incubate gels in gel drying solution for more than 20 minutes as the stained bands may fade.
- Discard the gel drying solution and cover the gel with a hard surface (a small petri dish or a glass plate) with light pressure on the gel surfaces **or** wrap the gel with two pieces of cellophane sheets to avoid curing during air-dry.
- Place in hood for air drying around 15-20 minutes **or** on bench for 1-2 hours **or** heat vacuum for 5-10 minutes and the gel is ready for storage.
  Note 1: Drying will take longer depending on humidity and gel thickness.
  Note 2: The temperature of the dried gel should be the same as the temperature of the surrounding gel drying surface. If the temperature of the dried gel is cooler, then the gel is not completely dried.
  Note 3: Check for moisture in the tubing connecting the gel dryer to the vacuum pump. The gel is not completely dried if there is any residual moisture in the tubing and additional drying time is required.
  Note 4: Wrap the dried gel with two pieces of cellophane sheets/plastic bags or one side with a filter paper, another side with a clear hard film. Press the dried gel(s) between the pages of a notebook under light pressure for gel storage.
- Photograph the dried-stained gel(s) and keep dry gel(s) in your notebook.

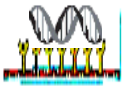
Flow Chart of electrophoretic mobility shift assay (EMSA):

1. **Prepartion of nucleic acid** and **protein samples** (50 minutes)
2. **EMSA binding reactions** (30 minutes) and **antibody binding reactions** (45 minutes)
3. **Native gel electrophoresis** and **"super-shift" detection** (30 minutes)
4. **Protein staining** (30 minutes) and **gel drying** (Optional)

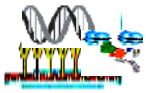
Nucleic acids (DNA/RNA/Oligo probes) **+**



Proteins (poly-peptides, purified or Nuclear proteins)

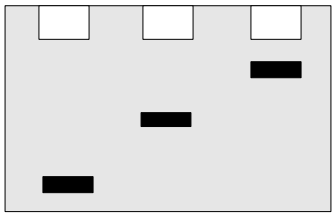


EMSA binding reactions (30 minutes) **to form** **Nucleic acid: Protein complex** **("gel-shift")**



Antibody binding reactions (45 minutes) **to form** **Nucleic acid: Protein: Antibody complex** **("super-shift")**

"super-shift" detection:



"super-shift" complex

"gel-shift" complex

"Free probes"

(4-6% native polyacrylamid gel)