

EMSA Accessory Kit

About the Kits

EMSA Accessory Kit 100 rxn 71282-3

Description

The EMSA Accessory Kit contains four key reagents needed to determine whether a specific DNA sequence binds to a protein in electrophoretic mobility shift assays (EMSA). The kit contains Poly(dI-dC)•Poly(dI-dC) solution, 4X EMSA Buffer, sonicated Salmon Sperm DNA and 100 mM DTT. Designed as a companion product to our NucBuster™ Protein Extraction Kit (Cat. No. 71183, see User Protocol TB338), the Novagen EMSA Accessory Kit is compatible with traditional methods of nuclear extract isolation and provides sufficient reagents to complete 100 reactions.

Components

- 1 ml 4X EMSA Buffer (400 mM KCl, 80 mM HEPES, 0.8 mM EDTA, 80% glycerol, pH 8.0)
- 125 µl Poly(dI-dC)•Poly(dI-dC) (0.01 U/µl in 100 mM KCl, 20 mM HEPES, pH 8.0)
- 150 µl Salmon Sperm DNA (500 ng/µl in nuclease-free water)
- 100 µl 100 mM DTT

Storage

Store all components at -20°C.

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Electrophoretic Mobility Shift Assay (EMSA)

DNA binding for EMSA

1. Combine 50 μ l 4X EMSA Buffer with 1 μ l 100 mM DTT. The final concentration of DTT in the protein/DNA complex reaction will be 0.5 mM.
2.

5 μ l	4X EMSA Buffer + DTT
1 μ l	500 ng/ μ l Sonicated Salmon Sperm DNA
1 μ l	Poly(dI-dC) \bullet Poly(dI-dC) (0.01 U)
x μ l	NucBuster™ extract. Typically 2.5 μ l is adequate. Adjust the amount according to the intensity of the shifted band.
<u>y</u> μ l	sterile water
17 μ l	total volume

Note: If non-specific binding is observed, increasing the amount of sonicated Salmon Sperm DNA and/or Poly(dI-dC) \bullet Poly(dI-dC) may reduce some background binding. Sufficient sonicated Salmon Sperm DNA and Poly(dI-dC) \bullet Poly(dI-dC) are provided in the kit to facilitate optimization.

3. Mix by pipetting up and down.
4. Add 1 μ l labeled probe (0.03 pmol) and mix.
5. Incubate on ice 30 min.

Note: If unlabeled specific competitor DNAs are used to establish specificity, mix the unlabeled and labeled DNA prior to adding to the extract. Adjust the total volume with the appropriate amount of water.

6. **Optional:** To identify complexed protein via an antibody “supershift,” add antibody to completed protein/DNA complex reaction. Incubate on ice for an additional 20 min before loading on gel.

Running DNA/protein complex on gels

1. Pre-run 6% non-denaturing DNA retardation gel (29:1 acrylamide to bisacrylamide) in 0.5X TBE (1 L = 5.4 g Tris base, 2.75 g Boric acid, 2 ml 0.5 M EDTA, pH 8) for 30 min at 100 V.
2. Add 2 μ l 6X DNA Loading Buffer (Cat. No. 69180-3) to 18 μ l DNA/protein complex and mix.
3. Load entire 20 μ l reaction in one well of DNA retardation gel.
4. Run at 100 V for 1 h or until the bottom dye has migrated to end of the gel.

Note: Running the gel for 1 h will likely leave uncomplexed probe still on the gel. If background is a problem, or if further separation between bands is required, run the gel for an additional 30 min.

5. Dry gel on DEAE paper using standard gel dryer.
6. Expose to autoradiographic film for appropriate length of time (4 h to overnight) at -80°C .

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Troubleshooting

Problem	Probable cause	Solution
No band shift detected on gel	Transcription factor of interest may not be expressed.	Raise expression levels within cell by induction (e.g., add phorbol ester to increase AP-1 expression) prior to extract preparation.
	Insufficient nuclear extract added.	Increase amount of nuclear extract added to binding reaction.
	Too much Salmon Sperm DNA or Poly(dI-dC)•Poly(dI-dC) added.	Lower concentration of non-specific competitors.
Too many shifted bands on gel	Transcription factor of interest may form multimeric binding complexes.	Establish specificity with antibody supershift assay.
	Non-specific DNA binding.	Increase concentration of non-specific competitors to decrease non-specific DNA binding.
Shifted bands appear blurry or faint on autoradiograph	DNA retardation gels may be too old.	Make fresh gels.
	Decayed probe.	Prepare fresh probe.

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