
Nucleolin EMSA Kit (Cat No. NS-001)

I. Reagents*

- A. 10x Reaction Buffer (250 μ l): 0.2 M Hepes-NaOH, pH 7.4, 10 mM DTT, 1 M NaCl.
- B. Nucleolin (50 μ l, 0.1 mg/ml)
- C. 5x Loading Buffer (0.5 ml)
- D. Protein Dilution Buffer (0.5 ml): 20 mM Hepes-NaOH, pH 7.4, 1 mM DTT, 0.5 mM PMSF, 50 mM NaCl, 0.1 mM EDTA, 0.05% NP-40, 0.2 mg/ml, and 20% glycerol.

**All reagents should be completely thawed, mixed by vortex, and briefly centrifuged. All reagents should be kept on ice during the preparation of reaction mixture described below and returned to below -70°C immediately after use.*

II. *In vitro* RNA binding assay

Step 1. Prepare master mix for N number of reactions (10 μ l/reaction)^a:

Reagent A	1x (N+1) μ l
³²P-RNA^b (10 fmol/μl)	1x (N+1) μ l
RNasin^b (1 unit/μl)	1x (N+1) μ l

Step 2. Adjust final volume of master mixture to 6x (N+1) μ l by adding deionized dH₂O^b.

Step 3. Transfer aliquot (6 μ l) to reaction tubes.

Step 4. Add competitor RNA or protein factor of your research interest up to 3 μ l.

Step 5. Add 0.2 to 1 μ l of nucleolin (**Reagent B**).

Step 6. Adjust the final reaction volume to 10 μ l by adding deionized dH₂O^b.

Step 7. Incubate at 25 to 37 $^{\circ}\text{C}$ for 15 min.

Step 8. Add 2.5 μ l aliquot of **Reagent C**.

Step 9. Resolve the entire mixture on a composite gel, as described in section III.

a. For reaction in higher volume, proportionally increase the amount of reagents described in this manual.

b. Not provided.

III. Native gel electrophoresis

Step 1. Cast a composite gel (30 ml) as follows at least 1 hr before sample loading (Notice; the smaller top glass plate should be siliconized and thoroughly washed before use):

30% polyacrylamide in ddH₂O	4.5 ml
5x TBE	3.0 ml
50% glycerol in ddH₂O	3.0 ml
ddH₂O	19.2 ml
10% ammonium persulfate	0.3 ml
TEMED	60 μ l

Step 2. Load the entire reaction mixture and perform electrophoresis at constant 14-21 mA.

Step 3. Remove the top glass plate and overlay the pre-cut filter paper on top of the gel.

Step 4. Turn the gel upside down and remove the bottom glass plate.

Step 5. Cover the gel with Syran wrap.

Step 6. Detect and quantify the RNA-nucleolin complex by autoradiography or by using PhosphorImager.