VAXRON

Nucleophosmin EMSA Kit (Cat No. BS-001)

I. Reagents*

A. 10x Reaction Buffer (250 μl): 0.2 M Hepes-NaOH, pH 7.4, 10 mM DTT, 1 M NaCl.
B. Nucleophosmin (50 μl, 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 μl/reaction)[a]:

Reagent A	1x (N+1) μl
^{32}P-RNA[b] (10 fmol/μl)	1x (N+1) μl
RNasin[b] (1 unit/μl)	1x (N+1) μl

Step 2. Adjust final volume of master mixture to 6x (N+1) μl by adding deionized dH_2O[b].
Step 3. Transfer aliquot (6 μl) to reaction tubes.
Step 4. Add competitor RNA or protein factor of your research interest up to 3 μl.
Step 5. Add 0.2 to 1 μl of nucleophosmin (**Reagent B**).
Step 6. Adjust the final reaction volume to 10 μl by adding deionized dH_2O[b].
Step 7. Incubate at 25 to 37°C for 15 min.
Step 8. Add 2.5 μl 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 ddH_2O	4.5 ml
5x TBE	3.0 ml
50% glycerol in ddH_2O	3.0 ml
ddH_2O	19.2 ml
10% ammonium persulfate	0.3 ml
TEMED	60 μl

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. Visualize and quantify the RNA-nucleophosmin complex by autoradiography or by using a PhosphorImager.

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For technical support, please contact us at support@vaxron.com.