

Product Information

NTF2-Agarose

Product Number **N 9285**

Storage Temperature 2-8 °C

Synonyms: Nuclear Transport Factor 2-agarose

Product Description

Nuclear transport factor 2 (NTF2) is a small homodimeric protein that interacts simultaneously with both Ran GDP and FxFG nucleoporins. The interaction between NTF2 and Ran-GDP is essential for the import of Ran into the nucleus, thus crucial for maintaining the cellular nuclear transport and cell viability.

NTF2-Agarose affinity resin is a useful tool for studying nuclear transport, and for depletion of Ran-GDP from cell extracts.¹⁻³

Reagent

NTF2-Agarose is supplied as 1:1 suspension in 1 M sodium chloride, containing 0.02% thimerosal.

Precautions and Disclaimer

This product is for laboratory research only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices

Storage/Stability

Store at 2-8 °C.

Procedure

Binding of Ran-GDP to NTF2-Agarose and analysis using 20% SDS-poly acrylamide gel (SDS-PAGE).

Note: NTF2 is a dimer. After addition of sample buffer to the conjugate, monomeric NTF2 may be released from the resin.

Reagents and Equipment

All the water used is 17 megohm grade

- NTF2 Agarose (Sigma product No. N 9285)
- Sepharose 4B control resin (Sigma product No. 4B-200)
- Ran (Sigma product no. R 3152)
- Assay buffer: 20 mM HEPES-KOH, pH 7.5, containing 110 mM potassium acetate, 2 mM magnesium acetate, 0.25 mM EGTA-KOH pH 7.5, 1 mM DTT, and 0.1% Tween® 20
- Microfuge tubes
- Table top microfuge centrifuge.

- Vertical rotating table
- Hamilton 27 gauge microsyringe. (50-100 µl)
- SDS-PAGE equipment
- 20% SDS-PAGE gel
- 2x sample buffer (Sigma product No. S 3401)
- Coomassie® blue stain.

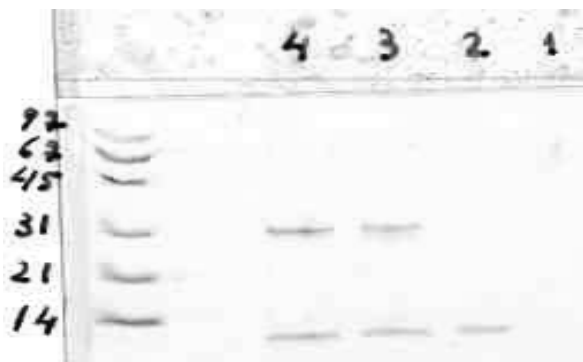
Reaction scheme

#	Sample	Sepharose 4B	NTF2-agarose 1:1 suspension	Assay buffer	Ran
1	Control 1	40 µl	-----	---	180 µl
2	Control 2	----	40 µl	180 µl	---
3	Sample 1	----	40 µl	---	180 µl
4	Sample 2	----	40 µl	---	180 µl

1. Take 1:1 suspension of Sepharose 4B and NTF2-agarose.
2. Dispense 40 µl of the 1:1 NTF2-agarose suspension into a microfuge tube for each assay (according to the reaction scheme above).
3. Dispense 40 µl of 1:1 suspension of Sepharose 4B to the control 1 tube.
4. Add 500 µl of assay buffer.
5. Centrifuge for 2 minutes at 2,000 x g.
6. Aspirate the buffer carefully.
7. Remove residual buffer using a Hamilton syringe.
8. Dilute the Ran to 0.1 mg/ml with assay buffer.
9. Add 180 µl of Ran solution to tubes # 1, 3, and 4 (approx. 18 µg protein).
10. Add 180 µl assay buffer to tube #2.
11. Rotate the tubes for 1 hour at room temperature.
12. Centrifuge the tubes (2,000 x g, 5 min.) and carefully aspirate the supernatant.
13. Wash each resin three times with assay buffer:
 - a. Add 500 µl assay buffer.
 - b. Centrifuge for 2 minutes at 2000 x g.
 - c. Aspirate the buffer carefully.
14. Remove the residual buffer using a Hamilton syringe.
15. Add 20 µl of 2x SDS-PAGE sample buffer to each pellet and boil for 5 min.

16. Centrifuge for 2 minutes at 2,000 x *g*.
17. Take the supernatant and run a 20% SDS-PAGE and stain with Coomassie® Blue.

Results



- Lane 1: Control resin
 Lane 2: NTF2-agarose without Ran
 Lane 3: NTF2-agarose with Ran
 Lane 4: Duplicate NTF2-agarose with Ran

The 30 kDa band is the Ran. The 12 kDa band is a NTF2 monomer.

References

1. Quimby, B. B., et al., Functional analysis of the hydrophobic patch on nuclear transport factor 2 involved in interactions with the nuclear pore in vivo. *J. Biol. Chem.*, **276**, 38820-38829 (2001).
2. Quimby, B. B., et al., The mechanism of ran import into the nucleus by nuclear transport factor 2. *J. Biol. Chem.*, **275**, 28575-28582 (2000).
3. Paschal, B.M., et al., Nucleotide-specific interaction of Ran/TC4 with nuclear transport factors NTF2 and p97. *Proc. Natl. Acad. Sci. USA*, **93**, 7679-83 (1996).

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