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ProductInformation

Nuclear Transport Factor 2
human, recombinant
expressed in *E. coli*

Product Number **N 4160**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Synonym: NTF2

Product Description
Nuclear Transport Factor 2 (NTF2) is a human, recombinant protein expressed in *E. coli*. It is a homodimeric protein containing 127 amino acids and the molecular weight of the monomer is approximately 15 kDa.

NTF2 mediates the nuclear import of Ran-GDP, driving the nuclear accumulation of Ran. This process is essential for maintaining cellular nuclear transport and for cell viability. NTF2, which possesses non-overlapping binding sites for both Ran-GDP and nucleoporin FxFG repeats, binds Ran-GDP in the cytoplasm and transports it via the nuclear pore complex (NPC) by binding to the FxFG repeats. The NTF2-[Ran-GDP] complex transport via the NPC is facilitated by the very low affinity of NTF2 to the FxFG repeats, which allows rapid attachment and detachment of NTF2 to the nucleoporins, while it passes through the NPC. At the nucleus side of the NPC, release of Ran-GDP from NTF2 may be promoted by dissociation of NTF2 into monomers.

This product is supplied as a lyophilized powder containing HEPES buffer salts, potassium acetate, magnesium acetate, EGTA, and sucrose.

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.

Preparation Instructions
Reconstitute the product with the lot specific volume of deionized water indicated on the product label. This results in a solution containing 20 mM HEPES, pH 7.5, 110 mM potassium acetate, 5 mM magnesium acetate, 0.5 mM EGTA, and 5% sucrose. The concentration of the NTF2 protein in the reconstituted solution is indicated on the product label.

Storage/Stability
The product ships on wet ice and it is recommended to store the product – 20 °C. Upon reconstitution, it is recommended to store the solution at –20 °C in working aliquots.

Procedure
The functionality of NTF2 is determined by measuring its binding to Ran. The binding assay is performed in two steps:
A.) binding of histidine-tagged Ran to EZview™ Red HIS-Select™ HC Nickel Affinity Gel
B.) binding of NTF2 to the Ran bound to the affinity resin. The NTF2-Ran complex is then analyzed by SDS-PAGE.

Reagents and equipment required:
- EZview™ Red HIS-Select™ HC Nickel Affinity Gel (Product No. E 3528)
- Ran (Product No. R 3152)
- NTF2 (Product No. N 4160)
- Assay Buffer: 20 mM HEPES, pH 7.5, 110 mM potassium acetate, 2 mM magnesium acetate, 0.25 mM EGTA, 1 mM DTT, and 0.1% TWEEN® 20
- Microcentrifuge tubes
- Table top microcentrifuge
- Vertical rotating table
- Hamilton® syringe (50-100 µl)
- Equipment and buffers for SDS-PAGE with Coomassie® Brilliant Blue Stain.

Reaction scheme

		Step 1			Step 2	
#		His-Select slurry	Ran	Assay buffer	NTF2	Assay buffer
1	Control 1	40 µl	____	160 µl	20 µg	complete to 200 µl
2	Control 2	40 µl	20 µg	complete to 200 µl	___	200 µl
3	Sample 1	40 µl	20 µg	complete to 200 µl	20 µg	complete to 200 µl
4	Sample 2	40 µl	20 µg	complete to 200 µl	20 µg	complete to 200 µl

Step A. Binding of RAN binding to EZview Red HIS-Select HC Nickel Affinity Gel

1. Each assay requires 40 µl of the 50% His-Select slurry. Calculate the volume of slurry required for the assays to be performed and add an additional 40 µl to the value to compensate for handling and pipetting loss. Add this volume of slurry to a microcentrifuge tube and mark the level of the slurry on the tube.
2. Centrifuge the tube to pellet the resin. Resuspend the pellet with 1 ml of assay buffer added to the microcentrifuge tube.
3. Repeat step 2 two more times. After the final wash, pellet the resin by centrifugation and add enough assay buffer to bring volume of the slurry up to the original level on the tube.
4. Dispense 40 µl of the His-Select slurry into a microcentrifuge tube for each assay.
5. Add 160 µl of assay buffer to the Control 1 tube. This control is the one without added Ran solution.
6. Add a volume of Ran solution, which is equivalent to 20 µg of protein, to the Control 2 tube and the Sample tubes.
7. Add sufficient assay buffer to each tube to bring the final volume to 200 µl.
8. Mix each tube for 2 hours at 4 °C in a vertical rotating table.
9. Centrifuge each tube and carefully aspirate the supernatant.
10. Wash each resin pellet three times with 0.5 ml of assay buffer. After the final wash, pellet the resin by centrifugation.

Step B. Binding of NTF2 to the Ran bound to the affinity resin.

1. Add a volume of NTF2 solution, which is equivalent to 20 µg of protein, to the resin pellets in Control 1 tube and the sample tubes. Do not add the NTF2 solution to the Control 2 tube.
2. Add sufficient assay buffer to each tube to bring the final volume to 200 µl.
3. Mix for 1 hour at room temperature using a vertical rotating table.
4. Centrifuge each tube and carefully aspirate the supernatant.
5. Wash each resin pellet three times with 0.5 ml of assay buffer.
6. After the final centrifugation, remove the residual buffer with a Hamilton syringe.
7. Add 20 µl of 2x SDS-PAGE sample buffer to each pellet and boil for 5 minutes.

Run the sample solutions on a 20% SDS-PAGE gel. Stain the gel with Coomassie Brilliant Blue. For samples containing NTF2, two bands are observed, one at approximately 15 kDa (NTF2) and the other at approximately 30 kDa (Ran).

References

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PY/MAM 11/02