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ProductInformation

RAN
Human recombinant, N-terminal histidine tagged, expressed in E.coli

Product Number **R 3152**
Storage Temperature –70 °C

Synonyms: TC4, Ras related nuclear protein

Product Description
Ran, a small GTP binding protein, is the heart of the nuclear transport process. It is involved in the directionality of the process that is regulated by GTP hydrolysis. Ran shuttles between the nucleus and the cytoplasm. In the nucleus it exists in the form of Ran-GTP, which is maintained by the Ran-Guanine nucleotide exchange protein, RCC1, a nucleus specific, chromatin bound protein. Ran-GTP forms complexes with transport proteins that shuttle from the nucleus to the cytoplasm, i.e. Importin β, CRM1/exportin1. In the cytoplasm, Ran intrinsic GTPase activity is stimulated by Ran-GAP causing the hydrolysis of GTP to GDP resulting in the uncoupling of Ran-GDP from the transport protein. Ran-GDP re-enters the nucleus by active transport as NTF2/Ran-GDP complex.

Ran is a unique small G-protein. Unlike many other small G-proteins, Ran possesses an acidic C-terminal tail that does not undergo post-translational lipid modification. This C-terminal tail regulates the interaction of Ran-GAP with Ran-GTP thus regulating the GTP hydrolysis.

Human Recombinant, N-terminal histidine tagged Ran is a 26.6 kDa protein. It appears on SDS-PAGE in low ionic strength buffer as a 31 kDa protein. In high ionic strength buffers it appears as a ladder of bands probably representing Ran oligomerization. Ran was purified in the presence of GDP.

Reagent
This product is supplied as a solution in
20 mM HEPES-potassium hydroxide,
110 mM potassium acetate, 2 mM magnesium cetate, 0.5 mM EGTA, 2 mM dithiothreitol (DTT), 5% glycerol pH 7.3.

Purity> 90% (SDS-PAGE)

Activity: Binds GDP at more than 0.1 molar ratio

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

Storage/Stability
The product ships on dry ice and storage at -70 °C is recommended.

Procedure: [^{3}H]GDP binding assay

Stock Solutions
- 0.5 M HEPES-Potassium hydroxide buffer (HEPES-KOH), pH 7.3
- 0.5 M Magnesium acetate ($MgAc_2$)
- 500 mM EDTA, pH 7.5
- 1 M KCl
- 4 M NaCl
- 1 mg/ml bovine serum albumin (BSA)
- 10 mM GDP, pH 7

Calibrated: molar extiction of GDP at 252 nm at pH 5.5 is 13,700. Dissolve 20 mg of GDP in 3 ml ddH_2O, titrate with 1 M NaOH to pH 7.0. Dilute a sample 1:200 in 0.1 M buffered acetate, pH 5.5 and measure OD at 252 nm. Calculate the concentration of the solution and correct the solution volume accordingly to give 10 mM GDP.
- 100 mM ATP, pH 7
- [3,8 -^{3}H]GDP - 1 mCi/ml, approx. 10 Ci/mmol

Working Solutions (for 50 tests)
- Enzyme Dilution Buffer,
  20 mM HEPES-KOH, 110 mM KAc, 2 mM $MgAc_2$, 0.5 mM EGTA, 2 mM DTT, 5% glycerol, pH 7.3, 1 ml
- 5X Reaction Buffer, 1 ml:
  100 mM HEPES, 2.5 mM DTT, 10 mM EDTA, 1 mM ATP, 400 mM GDP
- Stop Solution, 50 ml:
  20 mM HEPES, pH 7.3, 100 mM NaCl, 5 mM $MgAc_2$, 10 mg/ml BSA, pH 7.3.
- Wash solution, 1000 ml:
  20 mM HEPES, 100 mM NaCl, 5 mM $MgAc_2$, pH 7.3

Equipment
- Nitrocellulose filter disks, 0.45m, 25 mm diameter (Schleicher & Schuell NC-45 or equivalent)
- Vacuum manifold for 25 mm diameter filters dedicated to ^{3}H
- Scintillation vials
- Scintillation liquid

Reaction Scheme

	Ran	Enz.dil. Buffer	Reaction buffer 5X	[^{3}H]GDP	H_2O
	µl	µl	µl	µl	µl
Blank	---	X	10	1.5	38.5-X
Sample	X	----	10	1.5	38.5-X

1. Prepare the vacuum manifold according to manufacturerer's instructions using the nitrocellulose filters and putting the metal cylinders on top.
2. Prepare the Reaction Mixture consisting of the 5X Reaction Buffer, [^{3}H]GDP, and water. Prepare enough to run triplicates of each sample plus two spare reactions. (See Reaction Scheme above.) **Note:** If multiple Ran protein samples of different volumes are to be tested, then the water should be added separately (rather than as part of the 5x Reaction Mixture) prior to Step 5 such that the total reaction volume = 50 µl.
3. Add 12-15 µg Ran to the Sample microfuge tubes and add the same volume of Enzyme Dilution Buffer to the Blank tubes.
4. Add Reaction Mixture (total reaction volume should be 50 µl).
5. Incubate at 30 °C for 20 minutes.
6. Add 1 ml of Stop Solution to each tube.
7. Apply the vacuum on the vacuum manifold.
8. Filter each reaction mix through its own nitrocellulose filter.
9. Wash the filters four times with 5 ml each of wash solution. Apply vacuum until the filters are drained.
10. Transfer the filters to 7 ml scintillation vials.
11. Place 10 µl aliquots of each reaction mixture in two scintillation vials (for total CPM calculation).
12. Add 4 ml of scintillation liquid. Make sure that the filters are covered wth the liquid.
13. Incubate for at least 60 minutes.
14. Count radioactivity of ^{3}H in the scintillation counter.

Calculation

Given:
GDP per test = 4000 pmol
Ran MW= 26,600 (i.e. 26.6 µg =1000 pmoles)

Calculate:
Total cpm (T)
Specific radioactivity (SR), cpm/pmol= T/4000
Average sample and blank counts
Amount of Ran per test

$$pmolGDPincorporation = \frac{(CPM_{sample} - CPM_{blank})}{SR}$$

$$\%incorporation = \frac{pmolGDPincorporation}{pmolRanper\ test} \times 100$$

Results

Example:

GDP: 4000 pmol/test
Total cpm/test (T) : 1596532
Specific radioactivity (SR): 399 cpm/pmol
Ran: 1.6 mg/ml
Ran/test: 8 µl (= 12.8 µg or 481.2 pmol)

	cpm	cpm avg	cpm test - cpm blank	pmol GDP/ test	% incorporation
Blank	13884	11082			
	12401				
	6962				
Ran	54995	54954	42872	107	22
	45322				
	61547				

References

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3. Moore, S. and Blobel G., Nature, **365**, 661-663, 1993.
4. Conti, E. and Izaurralde, E., Curr. Opin. Cell. Biol., **13**, 310-319, 2001.

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