

**N-Ras Protein: wild-type  
(Human recombinant)**

**Cat. # CS-RS02**

**Lot # 011**

**Upon arrival store at 4°C (desiccated)**

**See datasheet for storage after reconstitution**

**Material**

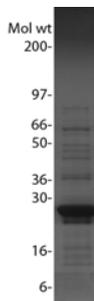
The wild-type human N-Ras protein has been produced in a bacterial expression system. The recombinant protein contains six histidine residues at its amino terminus (His-tag). The molecular weight of 6xHis tagged N-Ras is approximately 25 kDa and it is supplied as a white lyophilized powder.

**Storage and Reconstitution**

Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml with the addition of 20  $\mu$ l of Milli-Q water (100  $\mu$ g size). When reconstituted, the protein will be in the following buffer: 50 mM Tris pH 7.5, 50 mM NaCl, 0.5 mM MgCl<sub>2</sub>, 5% (w/v) sucrose, and 1% (w/v) dextran. In order to maintain high biological activity of the protein, it is strongly recommended that the protein solution be supplemented with DTT to 1 mM final concentration, aliquoted into "experiment-sized" amounts, snap frozen in liquid nitrogen, and stored at -70°C. The protein is stable for six months if stored at -70°C. **The protein should not be exposed to repeated freeze-thaw cycles.** The lyophilized protein is stable at 4°C desiccated (<10% humidity) for one year.

**Purity**

Protein purity is determined by scanning densitometry of Coomassie Blue-stained protein on a 4-20% polyacrylamide gradient gel. His tagged N-Ras protein was determined to be >90% pure. (see Figure 1).



**Figure 1. N-Ras Protein Purity Determination.** A 20  $\mu$ g sample of recombinant N-Ras protein (molecular weight approx. 25 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Life Technologies Inc.

**Biological Activity Assay**

The biological activity of N-Ras can be determined from the ability of the RasGRF1 exchange domain (Ras-GRF1-ExD) to catalyze the exchange of GDP for GTP on N-Ras. A standard biological assay for monitoring the biological activity of N-Ras is an exchange assay utilizing the 2X Exchange Buffer from the RhoGEF exchange assay kit (Cat.# BK100) and the human RasGRF GEF domain (Cat.# CS-GE03).

**Reagents**

1. Recombinant N-Ras protein (Cat.# CS-RS02)
2. Recombinant RAS-GRF GEF protein (Cat.# CS-GE03)
3. 2X Exchange Buffer (40 mM Tris pH 7.5, 100 mM NaCl, 20 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA, 1.5  $\mu$ M mant-GTP)
4. Dilution Buffer (20 mM Tris pH 7.5, 50 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA)

**Equipment**

1. Fluorescence spectrophotometer ( $\lambda$ ex=360 nm,  $\lambda$ em=440 nm)
2. Corning 96-well half area plates (Cat. # 3686) or other plate with low protein binding surface.

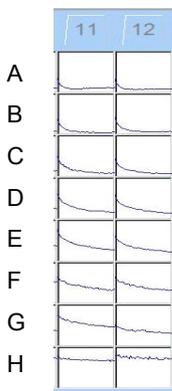
**Method**

1. Dilute Ras-GRF GEF protein (Cat.# CS-GE03) to 1  $\mu$ M (0.06 mg/ml) with Dilution Buffer.
2. Dilute N-Ras to 50  $\mu$ M (1.1 mg/ml) with Dilution Buffer.
3. Dissolve lyophilized 2X Exchange Buffer in 5 ml Milli-Q water and keep at room temperature.
5. Set up the plate reader for kinetic fluorescence measurements (Excitation wavelength at 360 nm and emission wavelength at 440 nm) with readings every 30 seconds for 30 minutes.
6. Add the following components together and mix well by gentle pipetting:

| <u>Exchange reaction mix</u> | <u>96 well black plate</u> |
|------------------------------|----------------------------|
| 2X Exchange Buffer           | 50 $\mu$ l                 |
| dH <sub>2</sub> O            | 26 $\mu$ l                 |
| 50 $\mu$ M N-Ras             | 4 $\mu$ l                  |

5. Pipette 20  $\mu$ l of 1  $\mu$ M Ras-GRF GEF protein or Dilution Buffer into their respective wells and immediately pipette up and down twice and begin reading the fluorescence.
6. Once the readings are complete and the plate reader file has been saved, the exchange rate can be calculated by reducing the data to Vmax with the software that accompanies the plate reader.

**Figure 2. Ras-GRF GEF protein mediated mant-GTP exchange on N-Ras.**



Legend: GDP-Bodipy-FL-loaded N-Ras protein was added to duplicate wells of a 96-well half area plate containing diluted Exchange Buffer and mixed well. To initiate the dissociation exchange reaction, 1 mM GTP plus Ras-GRF GEF protein (rows A to G), or 1 mM GTP only in Dilution Buffer (row H), was added to the wells, mixed, and fluorescence measurements were obtained using a Tecan SpectraFluor Plus Spectrophotometer. Ras-GRF was diluted 2 fold from row A through row G starting at 4  $\mu$ M. Note the rapid drop in fluorescence in rows A,B and C which is indicative of fast exchange, and the nearly flat line of row H which indicates no exchange is occurring in the absence of Ras-GRF.

**Product Uses**

- Study of N-Ras exchange activity with different GEFs
- Identification of N-Ras exchange factors (GEFs)
- Positive control for GEF studies
- Biochemical characterization of N-Ras protein interactions
- Western blot standard

**Product Citations/Related Products**

For the latest citations and related products, please visit [www.cytoskeleton.com](http://www.cytoskeleton.com).