

**RasGRF1 Protein: CDC25 domain (1038-1270) wild type
(Human recombinant, MBP tagged)**

Cat. # CS-GE03

Lot 011 Amount 1 x 100 µg

Upon arrival store at 4°C (desiccated)

See datasheet for storage after reconstitution

Background

RasGRF1 is a guanine exchange factor with selectivity for H-, K-, and N-Ras, which mediates Ras isoform activation under a variety of conditions and has been associated with multiple diseases (reviewed in 1, 2).

Material

The Cdc25 domain of human RasGRF1 protein has been produced in a bacterial expression system. It contains an MBP fusion at its amino terminus. The accession number is NM_002891.4. The molecular weight of MBP-RasGRF1 is approximately 60 kDa. The RasGRF1 protein is supplied as a white lyophilized powder. Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gradient gel. MBP-RasGRF1 was determined to be >85% pure. (see Figure 1).

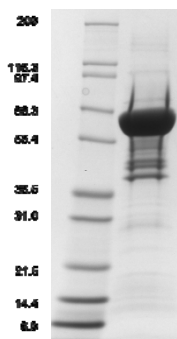


Figure 1. Purity Determination. A 20 µg sample of recombinant Ras-GRF1 (molecular weight approx. 60 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein was detected using the Precision Red Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Life Technologies Inc.

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 ice cold nanopure water (20 µl water per 100 µg protein). When reconstituted, the protein will be in the following buffer: 20 mM Tris pH 7.5, 50 mM NaCl, 1 mM MgCl₂, 5% (v/v) sucrose and 1% (v/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 must not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for one year. Further Ras-GRF1 dilutions should be made in 20 mM Tris pH 7.5, 50 mM NaCl, 1 mM MgCl₂. (not supplied).

Biological Activity Assay

The biological activity of RasGRF CDC25 domain can be determined from its ability to catalyze nucleotide exchange on Ras isoforms using the nucleotide exchange assay of Bodipy-FL-GDP by adding excess EDTA e.g. 0.7 mmol EDTA per mmol Mg2+ ions present in the reaction. This sub-stock solution is then used in a dissociation assay format which indicates competition for exchange site with unlabeled nucleotide. The reaction is monitored by fluorescence measurement at 485nm Ex / 535nm Em. Stringent quality control ensures that the exchange rate of Bodipy-GTP or mant-GTP is enhanced at least five fold in the presence of 0.8 µM RasGRF CDC25 domain .

Reagents and Equipment

1. MBP-RasGRF1 CDC25 domain protein (Cat. # CS-GE03).
2. Ras protein (Cat. # RS01 (H-Ras), RS02 (N-Ras), RS03 (K-Ras), RS04 (K-Ras-G12V), or RS05 (R-Ras)).
3. Exchange buffer 2 (20 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM DTT, 2 mM EDTA, 100 µg/ml BSA, and 0.75 µM Bodipy-GDP), note - make fresh.
4. 50 mM MgCl₂ in 20 mM Tris-HCl pH 7.5, 50 mM NaCl.
5. 5 mM GTP in 20 mM Tris-HCl pH 7.5, 50 mM NaCl..
6. 96-well plate fluorescence spectrophotometer.
7. Fluorescence half area low-binding black 96-well plate (Corning Cat. # 3686).

Method

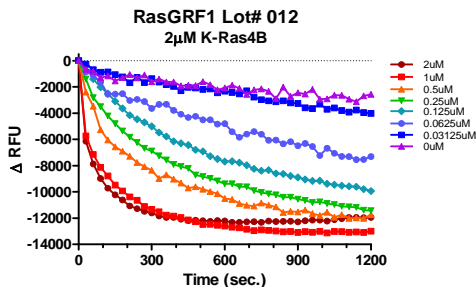
1. Place RasGRF vial on ice and dilute to 0.40 µg/µl (8 µM) with ice cold Exchange Buffer.
2. Dilute Ras protein to 1.25 µg/µl (50 µM) with ice cold Exchange Buffer..
3. Add the following components together in a fresh 15 ml Falcon tube and mix well by pipetting or gentle vortex:

Component	per well
Exchange Buffer	75 µl
50 µM Ras	5 µl
8 µM RasGRF	10 µl

Note: For a total mixture volume, multiply the volume of reagents per well by the number of wells in the experiment, plus add 20% volume for pipetting losses.

5. Incubate for 20 min at room temperature (RT).
6. Lock in the nucleotide by adding 10 µl (per well) of 50 mM MgCl₂.
7. Set up the fluorimeter with Excitation wavelength at 485 nm +/-20 nm and emission wavelength at 535 nm +/- 20 nm at RT.
8. Aliquot the pre-loaded mixture to the assigned wells and place the plate in the fluorimeter.
9. After 5 cycles (150 seconds), place the program on Hold or Pause, and remove the plate.
10. Pipette 10 µl of a) 5 mM GTP solution, b) a small compound, c) a test protein, d) 4 mM EDTA (+ve exchange control) or e) Dilution Buffer (negative control) in respective wells and immediately pipette up and down twice and resume reading for 20 minutes.
11. Save the readings after the kinetic protocols are finished. The exchange rate can be calculated by reducing the data to max slope (using 12 pts) or Vmax with the software that accompanies the plate reader. The exchange curve can be achieved by export to Microsoft Excel.

Figure 2. Ras-GRF1-catalyzed Bodipy-FL-GTP exchange assay.



Legend: .K-Ras4B protein (Cat. # RS03) (2.5 µM) was pre-loaded with Bodipy-FL-GDP using EDTA for exchange. The nucleotide was locked in place with excess Mg²⁺. RasGRF at different concentrations as shown or Dilution Buffer (purple) was pipetted into wells of a black 384-well low volume plate. At time zero, 500 µM GTP was pipetted in to the wells and the reactions were monitored for 20 min by reading every 30 sec.

References

1. Fernandez-Medarde A. and Santos E. 2011. *Biochim. Biophys. Acta.* 1815, 170-188.
2. Quilliam L.A. et al. 2002. *Prog. Nucleic Acid Res. Mol. Biol.* 71, 391-444.

Product Uses

- Study of RasGRF1 interacting proteins, such as H-, K-, N- and R-Ras GTPases.
- Identification of RasGRF1 interacting proteins.
- Drug discovery tool for RasGRF1/Ras pathway inhibitors.
- Positive control for Western blots.

Product Citations/Related Products

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