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Catalog #	Aliquot Size
G127-310G-05	05 µg
G127-310G-10	10 µg

GNAS, Active

Full-length human recombinant protein expressed in E.coli cells

Catalog # G127-310G

Lot # L2195-7

Product Description

Full-length recombinant human GNAS was expressed in E.coli cells using an N-terminal GST tag. The gene of GNAS accession number is NM_000516.

Gene Aliases

AHO; C20orf45; GNAS1; GPSA; GSA; GSP; NESP; POH; SCG6; SgVI

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 50mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

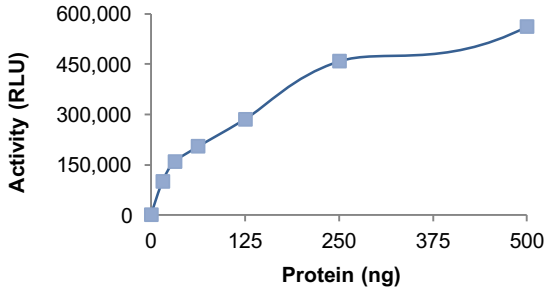
Scientific Background

Guanine nucleotide-binding proteins (G proteins) are heterotrimeric signal-transducing molecules consisting of alpha, beta, and gamma subunits, and function as transducers downstream of G protein-coupled receptors (GPCRs) in numerous signaling cascades. The alpha subunit binds guanine nucleotide, can hydrolyze GTP, and can interact with other proteins. Guanine nucleotide-binding protein G(s) subunit alpha isoforms short (GNAS) functions downstream of several GPCRs, including beta-adrenergic receptors. Stimulates the Ras signaling pathway via RAPGEF2.

References

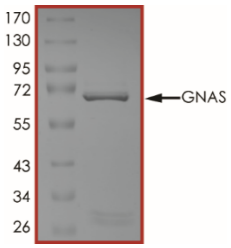
1. Thiele S, et al: Functional characterization of GNAS mutations found in patients with pseudohypoparathyroidism type Ic defines a new subgroup of pseudohypoparathyroidism affecting selectively Gsa-receptor interaction. Hum Mutat. 2011 Jun;32(6):653-60.
2. Pak Y, et al: Direct binding of the beta1 adrenergic receptor to the cyclic AMP-dependent guanine nucleotide exchange factor CNrasGEF leads to Ras activation. Mol Cell Biol. 2002 Nov;22(22):7942-52.
3. http://www.uniprot.org/uniprot/P63092

Specific Activity



The specific activity of GNAS was determined to be **38 nmol/min/mg** as per activity assay protocol.

Purity



The purity of GNAS was determined to be **>95%** by densitometry.
Approx. MW **70kDa**.

GNAS, Active

Full-length human recombinant protein expressed in E.coli cells

Catalog #	G127-310G
Specific Activity	38 nmol/min/mg
Lot #	L2195-7
Purity	>95%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at the recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active GNAS (Catalog #: G127-310G)

Active GNAS (0.1μg/μl) diluted with GTPase/GAP Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active GNAS for optimal results).

GTPase-Glo™ Assay (Promega, Catalog# V7681)

GTPase/GAP Buffer, 5ml
GEF Buffer, 5ml
GTPase-Glo™ Buffer, 5ml
GTPase-Glo™ Reagent, 500X, 15μl
ADP, 10mM, 0.5ml
Detection Reagent, 10ml
rGTP, 10mM, 50μl
DTT, 100mM, 0.1ml

Assay Protocol

The GTPase assay is performed using the GTPase-Glo™ Assay kit (Promega), by detecting the amount of GTP remaining after GTP hydrolysis in a GTPase reaction. The remaining GTP is converted to ATP using the GTPase-Glo™ Reagent, and the ATP is then detected using a thermostable luciferase and luciferin substrate to produce bioluminescence. GTPase activity is inversely correlated to the amount of light produced.

Step 1. Thaw the active GNAS on ice and prepare the following working solutions with GTPase/GAP Buffer:

- 2X final concentration of Active GNAS (Catalog #: G127-310G)
- 2X GTP solution containing 2μM GTP and 2mM DTT

Step 2. In a polystyrene 96-well plate, add the following components to bring the initial reaction volume to 20 μl:

Component 1. 10 μl of 2X Active GNAS (Catalog #: G127-310G)
Component 2. 10 μl of 2X GTP solution

Note: A blank control can be set up as outlined in step 2 by replacing the enzyme working solution with an equal volume of GTPase/GAP Buffer.

Step 3. Mix the reaction on an orbital shaker for 2 minutes. Incubate the reaction at room temperature for the optimal time, generally 60 minutes.

Step 4. Prepare the required volume of reconstituted GTPase-Glo™ Reagent (1X) containing 5μM ADP with GTPase-Glo™ Buffer, equilibrate to room temperature before use.

Step 5. Add 20μl of reconstituted GTPase-Glo™ Reagent to the completed GTPase reactions, mix briefly and incubate with shaking at room temperature for 30 minutes.

Step 6. Add 40μl of Detection Reagent and incubate the plate for 5-10 minutes at room temperature.

Step 7. Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).

Step 8. Determine the corrected activity (RLU) by removing each sample's value from the blank control (see Step 2) and calculate the GTPase specific activity as outlined below.

Calculation of GTP Specific Activity (SA) (RLU/pmol)

Specific activity (SA) = RLU of the blank control / pmoles of GTP in the blank control
(i.e., 10μl * 1μM GTP * 10^{-6} = 10 μmols * 10^{-6} = 10 pmols)

GTPase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected RLU from reaction / [(SA of GTP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]

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