

Active Recombinant Human NRAS Protein, His-tagged

Cat. No. NRAS-01H **Lot. No.** (See product label)

SPECIFICATION

Product Overview	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).
Species	Human
Source	Bacteria
Description	This is an N-ras oncogene encoding a membrane protein that shuttles between the Golgi apparatus and the plasma membrane. This shuttling is regulated through palmitoylation and depalmitoylation by the ZDHHC9-GOLGA7 complex. The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein. Mutations in this gene have been associated with somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, Noonan syndrome, and juvenile myelomonocytic leukemia.
Form	White lyophilized powder
Bio-activity	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 2× Exchange Buffer from the RhoGEF exchange assay kit and the human RasGRF GEF domain.

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Molecular Mass	25 kDa
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.
Applications	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
Stability	The protein is stable for six months if stored at -70 centigrade. The protein should not be exposed to repeated freezethaw cycles. The lyophilized protein is stable at 4 centigrade desiccated (<10% humidity) for one year.
Storage	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 centigrade.
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 µL of Milli-Q water (100 µg size). When reconstituted, the protein will be in the following buffer: 50 mM Tris pH 7.5, 50 mM NaCl, 0.5 mM MgCl ₂ , 5% (w/v) sucrose, and 1% (w/v) dextran.

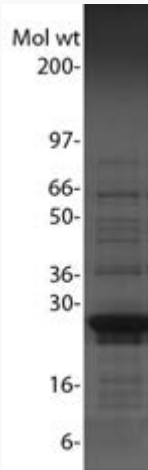
GENE INFORMATION

Gene Name	NRAS NRAS proto-oncogene, GTPase [Homo sapiens (human)]
Official Symbol	NRAS

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Synonyms	NRAS; NRAS proto-oncogene, GTPase; NS6; CMNS; NCMS; ALPS4; N-ras; NRAS1; GTPase NRas; N-ras protein part 4; neuroblastoma RAS viral (v-ras) oncogene homolog; neuroblastoma RAS viral oncogene homolog; transforming protein N-Ras; v-ras neuroblastoma RAS viral oncogene homolog; EC 3.6.5.2
Gene ID	4893
mRNA Refseq	NM_002524
Protein Refseq	NP_002515
MIM	164790
UniProt ID	P01111
Purity	 A 20 µg 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. Mark12 molecular weight markers are from Life

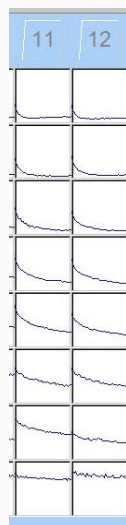
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Technologies Inc.

Ras-GRF GEF protein mediated Bodipy-GDP dissociation/exchange on N-Ras.



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 μ 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.

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