

PTC3 (NCOA4-RET), Active

Recombinant human fusion protein expressed in Sf9 cells

Catalog # R02-19DG

Lot # G1234-4

Product Description

Recombinant human RET/PTC3, the fusion protein [NCOA4 (1-238)-RET (713-end)], was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The NCOA4 gene accession number is [BC001562](#) and RET is [NM_020630](#).

Gene Aliases

NCOA4: ELE1, RFG, ARA70, PTC3, DKFZp762E1112
RET: CDHF12, RET51, PTC, RET-ELE1; RET/PTC3

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM 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.

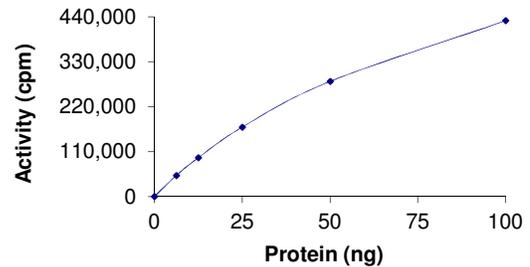
Scientific Background

RET/PTC3 is fused of RET and the activating NCOA4 gene by intrachromosomal paracentric inversions in chromosome 10 (1). Like RET/PTC1, it is the most frequent RET rearrangements in papillary thyroid carcinoma (PTC) (2), especially in radiation-induced tumours. The RET/PTC3 rearrangements may be typical for radiation-induced childhood PTC with a short latency period (3). The RET/PTC rearrangements also have been shown in benign thyroid lesions, including Hashimoto's thyroiditis (HT).

References

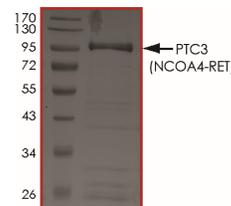
1. Santoro M, et al: Molecular characterization of RET/PTC3; a novel rearranged version of the RET proto-oncogene in a human thyroid papillary carcinoma. *Oncogene* 1994, 9:509-516.
2. Nikiforov YE: RET/PTC rearrangement in thyroid tumors. *Endocr Pathol* 2002, 13:3-16.
3. Smida J. et al: Distinct frequency of ret rearrangements in papillary thyroid carcinomas of children and adults from Belarus. *Int J Cancer* 1999, 80:32-38.

Specific Activity



The specific activity of PTC3 (NCOA4-RET) was determined to be **285 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PTC3 (NCOA4-RET) was determined to be **>80%** by densitometry, approx. MW **98 kDa**.

PTC3 (NCOA4-RET), Active

Recombinant human fusion protein expressed in Sf9 cells

Catalog #	R02-19DG
Specific Activity	285 nmol/min/mg
Lot #	G1234-4
Purity	>80%
Concentration	0.1 µg/µl
Stability	1 yr 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 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 Kinase (Catalog #: R02-19DG)

Active PTC3 (NCOA4-RET) (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) 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 PTC3 (NCOA4-RET) for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: I15-58)

IGF1Rtide synthetic peptide substrate (KKKSPGEYVNIEFG) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active PTC3 (NCOA4-RET), Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1.** 10µl of diluted Active PTC3 (NCOA4-RET) (Catalog #R02-19DG)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #I15-58)
 - Component 3.** 5µl distilled H₂O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 5.** Initiate the reaction by the addition of 5 µl [³³P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H₂O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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