

RET (Y791F), Active

Recombinant human mutant protein expressed in Sf9 cells

Catalog # R02-12CG-10

Lot # F685-1

Product Description

Recombinant human RET (Y791F) (658-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The RET gene accession number is [NM_020630](#).

Gene Aliases

PTC, MTC1, HSCR1, MEN2A, MEN2B, RET51, CDHF12, RET-ELE1

Concentration

0.1 µg/µl

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, Shipping 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. Stability is 1yr at -70°C from date of shipment. Product shipped on dry ice.

Scientific Background

RET or ret proto-oncogene is a member of the cadherin superfamily that encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation. RET can undergo oncogenic activation in vivo and in vitro by cytogenetic rearrangement (1). Mutations in the RET gene are associated with the disorders multiple endocrine neoplasia, type IIA, multiple endocrine neoplasia, type IIB, Hirschsprung disease, and medullary thyroid carcinoma. RET signaling pathway, by regulating the development of both the nervous and lymphoid system in the gut, plays a key role in the molecular mechanisms that orchestrate intestine organogenesis (2).

References

1. Grieco, M. et.al: PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. *Cell* 60: 557-563, 1990.
2. Veiga-Fernandes, H. et.al: Tyrosine kinase receptors RET is a key regulator of Peyer's patch organogenesis. *Nature* 446: 547-551, 2007.

Purity

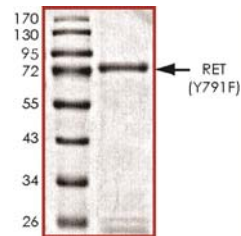
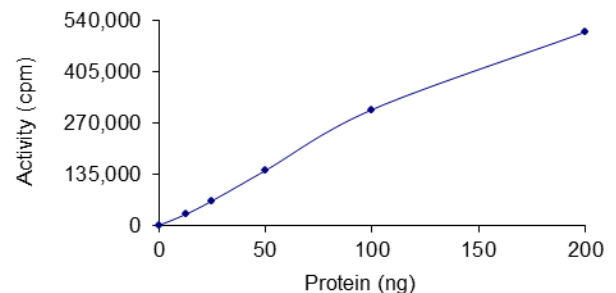


Figure 1. SDS-PAGE gel image

The purity of RET (Y791F) was determined to be **>95%** by densitometry, approx. MW **~74kDa**.

Specific Activity

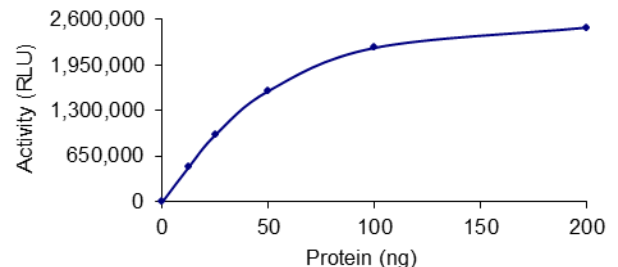
Figure 2. Radiometric Assay Data



The specific activity of RET (Y791F) was determined to be **98 nmol /min/mg** as per activity assay protocol.

(For Radiometric Assay Protocol on this product please see pg. 2)

Figure 3. ADP-Glo™ Assay Data



The specific activity of RET (Y791F) was determined to be **720 nmol /min/mg** as per activity assay protocol.

(For ADP-Glo™ Assay Protocol on this product please see pg. 3)

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: R02-12CG)

Active RET (Y791F) (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 RET (Y791F) 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 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 RET (Y791F), 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 RET (Y791F) (Catalog # R02-12CG)
 - 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 5µl [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 20µl 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 5µl [³³P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1,250 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)]

ADP-Glo™ Activity Assay Protocol

Reaction Components

RET (Y791F) Kinase Enzyme System (Promega, Catalog #:V5326)

RET (Y791F), Active, 10µg (0.1µg/µl)
IGF1Rtide substrate, 1ml (1mg/ml)
Reaction Buffer A (5X), 1.5ml
DTT solution (0.1M), 25µl

ADP-Glo™ Kinase Assay Kit (Promega, Catalog #: V9101)

Ultra Pure ATP, 10 mM (0.5ml)
ADP, 10 mM (0.5ml)
ADP-Glo™ Reagent (5ml)
Kinase Detection Buffer (10ml)
Kinase Detection Substrate (Lyophilized)

Reaction Buffer A (5X)

200mM Tris-HCl, pH 7.5, 100mM MgCl₂ and 0.5 µg/µl BSA.

Assay Protocol

The RET (Y791F) assay is performed using the RET (Y791F) Kinase Enzyme System (Promega; Catalog #: V5326) and ADP-Glo™ Kinase Assay kit (Promega; Catalog #: V9101). The RET (Y791F) reaction utilizes ATP and generates ADP. Then the ADP-Glo™ Reagent is added to simultaneously terminate the kinase reaction and deplete the remaining ATP. Finally, the Kinase Detection Reagent is added to convert ADP to ATP and the newly synthesized ATP is converted to light using the luciferase/luciferin reaction. For more detailed protocol regarding the *ADP-Glo™ Kinase Assay*, see the Technical Manual #TM313, available at www.promega.com/tbs/tm313/tm313.html.

- Step 1.** Thaw the ADP-Glo™ Reagents at ambient temperature. Then prepare Kinase Detection Reagent by mixing Kinase Detection Buffer with the Lyophilized Kinase Detection Substrate. Set aside.
- Step 2.** Thaw the components of RET (Y791F) Enzyme System, ADP and ATP on ice.
- Step 3.** Prepare 1ml of 2X Buffer by combining 400µl Reaction Buffer A, 1µl DTT and 599µl of dH₂O.
- Step 4.** Prepare 1ml of 250µM ATP Assay Solution by adding 25µl ATP solution (10mM) to 500µl of 2X Buffer and 475µl of dH₂O.
- Step 5.** Prepare diluted RET (Y791F) in 1X Buffer (diluted from 2X buffer) 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 RET (Y791F) for optimal results).
- Step 6.** In a white 96-well plate (Corning Cat # 3912), add the following reaction components bringing the initial reaction volume up to 20µl:

Component 1.	10µl of diluted Active RET (Y791F)
Component 2.	5µl of 1mg/ml stock solution of substrate
Component 3.	5µl of 2X Buffer
- Step 7.** Set up the blank control as outlined in step 6, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H₂O.
- Step 8.** At the same time as the RET (Y791F) kinase reaction, set up an ATP to ADP conversion curve at 50µM ATP/ADP range as described in the *ADP-Glo™ Kinase Assay* Technical Manual #TM313.
- Step 9.** Initiate the RET (Y791F) reactions by the addition of 5µl of 250µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the plate and incubate the reaction mixture at 30°C for 15 minutes.
- Step 10.** Terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minute at ambient temperature.
- Step 11.** Add 50µl of the Kinase Detection Reagent, shake the plate and then incubate the reaction mixture for another 30 minute at ambient temperature.
- Step 12.** Read the 96-well reaction plate using the Kinase-Glo™ Luminescence Protocol on a GloMax® Microplate Luminometer (Promega; Cat # E6501).
- Step 13.** Using the conversion curve, determine the amount of ADP produced (nmol) in the presence (step 6) and absence of substrate (Step 7) and calculate the kinase specific activity as outlined below. For a detailed protocol of how to determine nmols from RLUs, see ADP-Glo™ Applications Database at <http://www.promega.com/applications/cellularanalysis/cellsignaling.htm>

Kinase Specific Activity (SA) (nmol/min/mg)

(ADP (step 6) – ADP (Step 7)) in nmol) / (Reaction time in min)*(Enzyme amount in mg)