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Product Information

RET (V804L), active, GST-tagged, human
PRECISIO® Kinase
recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5311**
Storage Temperature –70 °C

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

Product Description

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. The 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]

Recombinant human RET (V804L) (658-end) was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST-tag. The RET gene accession number is NM_020630. It is supplied in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~74 kDa

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot:
≥70% (SDS-PAGE, densitometry)

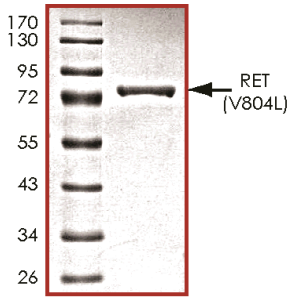
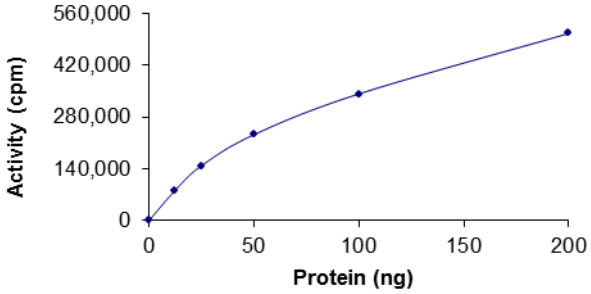


Figure 2.
Specific Activity of Typical Lot:
364–546 nmole/min/mg



Procedure

Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7. 2, 12.5 mM glycerol 2-phosphate, 25 mM $MgC1_2$, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA solution.

Kinase Solution – Dilute the active RET (0.1 μg/μL) with Kinase Dilution Buffer to the desired concentration. Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active RET kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 mL of Kinase Assay Buffer. Store in 200 μL aliquots at –20 °C.

γ-^{33}P-ATP Assay Cocktail (250 μM) – Combine 5.75 mL of Kinase Assay Buffer, 150 μL of 10 mM ATP Stock Solution, 100 μL of γ-^{33}P-ATP (1 mCi/100 μL). Store in 1 mL aliquots at –20 °C.

Substrate Solution – IGF1Rtide peptide substrate (KKKSPGEYVNIEFG) diluted in distilled water to a final concentration of 1 mg/mL.

1% phosphoric acid solution – Dilute 10 mL of concentrated phosphoric acid to a final volume of 1 L with water.

Kinase Assay

This assay involves the use of the ^{33}P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active RET, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-^{33}P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 μL:
   - 10 μL of Kinase Solution
   - 5 μL of Substrate Solution
   - 5 μL of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5 μL of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 μL of the γ-^{33}P-ATP Assay Cocktail, bringing the final reaction volume to 25 μL. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 μL of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-^{33}P-ATP counts introduced into the reaction. Spot 5 μL of the γ-^{33}P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

Calculations:

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

$$SR = \frac{\text{cpm of 5 μL of γ-}^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

   cpm – value from control (step 7)
   nmole – 1.25 nmole (5 μL of 250 μM ATP Assay Cocktail)

2. Specific Kinase Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta\text{cpm} \times (25/20)}{SR \times E \times T}$$

SR = specific radioactivity of the ATP (cpm/nmole ATP)
Δcpm = cpm of the sample – cpm of the blank (step 3)
25 = total reaction volume
20 = spot volume
T = reaction time (minutes)
E = amount of enzyme (mg)

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).

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