Product Information

RET (V804L), active, GST-tagged, human PRECISIO® Kinase recombinant, expressed in Sf9 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 Sf9 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.

Procedure
Preparation Instructions
Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgC12, 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-33P-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-33P-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 33P 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-33P-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-33P-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-33P-ATP counts introduced into the reaction. Spot 5 µL of the γ-33P-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)
   \[
   SR = \frac{\text{cpm of 5 µL of } \gamma^{33}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}
   \]
   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)
   \[
   \text{nmole/min/mg} = \frac{\Delta \text{cpm} \times (25/20)}{\text{SR} \times E \times T}
   \]
   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**


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