

# Product Information

## RET (658-end), active, GST-tagged, human Precisio® Kinase recombinant, expressed in Sf9 cells

Catalog Number **R7782**

Lot Number 030M0798

Storage Temperature –70 °C

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

### Product Description

The *RET* gene codes for a transmembrane tyrosine kinase, which is a subunit of a multimeric complex that acts as a receptor for four structurally related molecules: GDNF, neurturin, artemin, and persephin.<sup>1</sup> Germline mutations of *RET* cause a dominantly inherited dysgenesis of the enteric nervous system known as Hirschsprung's disease. *RET* is constitutively activated by point mutations in hereditary medullary thyroid carcinomas (MTC). Several single nucleotide polymorphisms of the *RET* gene have been described. Multiple endocrine neoplasia type 2A (MEN 2A) has been reported to be associated with two mutations of the protooncogene *RET*.<sup>2</sup>

This recombinant product was expressed by baculovirus in *Sf9* insect cells using an N-terminal GST-tag. The gene accession number is NM 020630. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~74 kDa

Purity: ≥70% (SDS-PAGE, see Figure 1)

Specific Activity: 57–78 nmole/min/mg (see Figure 2)

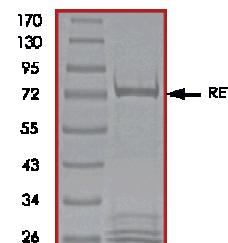
### Precautions and Disclaimer

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.

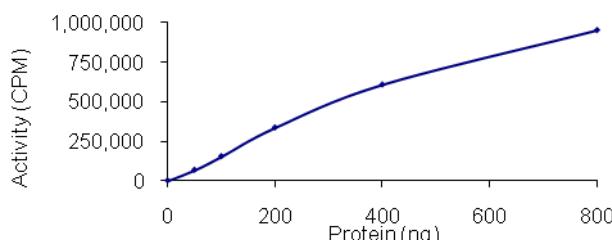
### Storage/Stability

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 Lot Number 030M0798:  
>80% (densitometry)



**Figure 2.**  
Specific Activity of Lot Number 030M0798:  
74 nmole/min/mg



### Procedure

#### Preparation Instructions

Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl<sub>2</sub>, 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 RET, active (0.1  $\mu$ g/ $\mu$ l) with Kinase Dilution Buffer to the desired concentration.

Note: The lot-specific specific activity plot may be used as a guideline (see Figure 2). It is recommended that the researcher perform a serial dilution of RET, active for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200  $\mu$ l aliquots at –20 °C.

$\gamma$ -<sup>32</sup>P-ATP Assay Cocktail (250  $\mu$ M) – Combine 5.75 ml of Kinase Assay Buffer, 150  $\mu$ l of 10 mM ATP Stock Solution, 100  $\mu$ l of  $\gamma$ -<sup>32</sup>P-ATP (1 mCi/100  $\mu$ l). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate (KKKSPGEYVNIEFG) in water at 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 <sup>32</sup>P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the RET, active, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The  $\gamma$ -<sup>32</sup>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  $\mu$ l:  
10  $\mu$ l of Kinase Solution  
5  $\mu$ l of Substrate Solution  
5  $\mu$ l of cold water (4 °C)
3. Set up a blank control as outlined in step 2, substituting 5  $\mu$ l of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5  $\mu$ l of the  $\gamma$ -<sup>32</sup>P-ATP Assay Cocktail, bringing the final reaction volume to 25  $\mu$ l. 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  $\mu$ l of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.

6. Air dry the pre-cut 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  $\gamma$ -<sup>32</sup>P-ATP counts introduced into the reaction. Spot 5  $\mu$ l of the  $\gamma$ -<sup>32</sup>P-ATP Assay Cocktail on a pre-cut 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)

$$SR = \frac{\text{cpm of } 5 \mu\text{l of } \gamma\text{-}^{32}\text{P-ATP Assay Cocktail}}{\text{nmole of ATP}}$$

cpm – value from control (step 7)

nmole – 1.25 nmole (5  $\mu$ l of 250  $\mu$ M 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)

$\Delta$ cpm = 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. Geneste, O. et al., Two distinct mutations of the RET receptor causing Hirschsprung's disease impair the binding of signalling effectors to a multifunctional docking site. *Hum. Mol. Genet.*, **8**, 1989-1999 (1999).
2. Kahn, T.A. et al., A novel case of multiple endocrine neoplasia type 2A associated with two *de novo* mutations of the RET protooncogene. *J. Clin. Endocrinol. Metab.*, **84**, 3522-3527 (1999).

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