

## Product Information

**COT (30-397), active, GST-tagged, human  
PRECISIO® Kinase  
recombinant, expressed in *Sf9* cells**

Catalog Number **C1370**  
Lot Number SLBB7131V  
Storage Temperature  $-70^{\circ}\text{C}$

Synonyms: MAP3K8, EST, ESTF, TPL2, Tpl-2, c-COT,  
FLJ10486

### Product Description

COT is an oncogene that can activate both the MAP kinase and JNK kinase pathways. COT activates I $\kappa$ B kinases and induces the nuclear production of NF- $\kappa$ B. C-terminal catalytic domain of KSR2 associates with COT and KSR2 can negatively regulate the kinase activity of COT *in vitro*. Co-transfection of KSR2 with COT in cells leads to reduced COT-mediated ERK activation and COT-induced IL-8 production in a dose-dependent manner.<sup>1</sup> COT is one of the MAP kinase kinase kinases that regulates the ERK1/ERK2 pathway in response to IL-1. Blockage of expression of COT results in failure of IL-1 to induce an increase in IL-8 and MIP-1 $\beta$  mRNA levels.<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 005204. 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: ~70 kDa

Purity:  $\geq 70\%$  (SDS-PAGE, see Figure 1)

Specific Activity: 860–1,164 nmole/min/mg  
(see Figure 2)

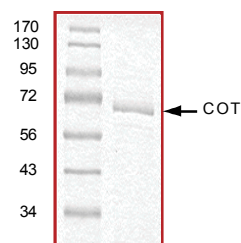
### Precautions and Disclaimer

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.

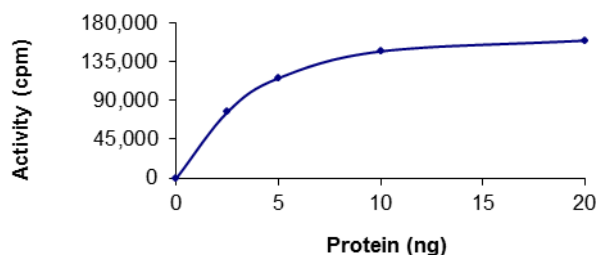
### Storage/Stability

The product ships on dry ice and storage at  $-70^{\circ}\text{C}$  is recommended. After opening, aliquot into smaller quantities and store at  $-70^{\circ}\text{C}$ . Avoid repeated handling and multiple freeze/thaw cycles.

**Figure 1.**  
SDS-PAGE Gel of Lot Number SLBB7131V:  
>90% (densitometry)



**Figure 2.**  
Specific Activity of Lot Number SLBB7131V:  
1150 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.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 μl aliquots at  $-20^{\circ}\text{C}$ .

Kinase Solution – Dilute the active COT (0.1 µg/µl) 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 the researcher perform a serial dilution of active COT kinase for optimal results.

$\gamma$ -<sup>32</sup>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  $\gamma$ -<sup>32</sup>P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Inactive MEK1 (0.2 µg/µl), Inactive ERK1 (0.2 µg/µl), Myelin Basic Protein (MBP) diluted 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 active COT, Kinase Assay Buffer, Inactive MEK1, Inactive ERK1, 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, prepare an activation mixture with a final volume of 20 µl:
  - 10 µl of Kinase Solution
  - 2 µl of inactive MEK1 (0.2 µg/µl)
  - 3 µl of inactive ERK1 (0.2 µg/µl)
  - 5 µl of Kinase Dilution Buffer
3. Start the activation reaction by adding 5 µl of 250 µM ATP and incubate in a water bath at 30 °C for 25 minutes.
4. In a microcentrifuge tube, add the following solutions to a volume of 20 µl on ice:
  - 5 µl of the activated mixture (step 3)
  - 5 µl of MBP Substrate Solution (4 °C)
  - 10 µl of cold water (4 °C)
5. Set up a blank control as outlined in step 4, substituting 5 µl of cold water (4 °C) for the Substrate Solution.
6. Initiate each reaction with the addition of 5 µl of the  $\gamma$ -<sup>32</sup>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.

7. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually pre-cut strip of phosphocellulose P81 paper.
8. 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.
9. Set up a radioactive control to measure the total  $\gamma$ -<sup>32</sup>P-ATP counts introduced into the reaction. Spot 5 µl 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.
10. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
11. 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)

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

$$\begin{aligned} \text{cpm} &= \text{value from control (step 7)} \\ \text{nmole} &= 1.25 \text{ nmole (5 } \mu\text{l of 250 } \mu\text{M ATP Assay Cocktail)} \end{aligned}$$

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)

$\Delta\text{cpm}$  = 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. Channavajhala, P.L. et al., J. Biol. Chem., **278**, 47089-47097 (2003).
2. Rodríguez, C. et al., Cell Signal., **18**, 1376-1385 (2006).

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