Recombinant Human Active MAP3K8/Tpl2/COT Protein, CF

Catalog Number: 4586-KS-010
Lot Number: 1655697

SPECIFICATIONS AND USE

Source	Recombinant human COT (amino acids 30-397) was expressed by baculovirus in *Sf* 9 insect cells using an N-terminal GST tag. The gene accession number is NM_005204.
Gene Aliases	MAP3K8, EST, ESTF, TPL2, Tpl-2, c-COT, FLJ10486.
Molecular Mass	The approximate molecular weight is 70 kDa.
Purity	The purity was determined to be > 90% by densitometry.
Formulation	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, 25% Glycerol.
Size	10 µg
Concentration	0.1 µg/µL
Activity	The specific activity of this lot of COT was determined to be 21 nmol/min/mg as per the Activity Assay Protocol.
Storage	This product is stable at ≤ -70 °C for up to one year from the date of receipt. For optimal storage, aliquot into smaller quantities after centrifugation and store at recommended temperature. **Avoid repeated freeze-thaw cycles.**

BACKGROUND

COT is an oncogene that can activate both the MAP kinase and JNK kinase pathways. COT activates IκB kinases and induces the nuclear production of NF-κB. The 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 lead to reduced COT-mediated ERK activation and COT induced IL-8 production in a dose-dependent manner (1). COT is one of the MAP kinase kinase (MAPKK) kinases that regulates the ERK1/COT 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-1β mRNA levels (2).

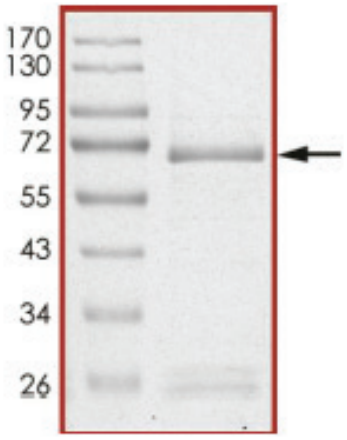

Figure 1: The purity of COT was determined to be > 90% by densitometry. Approximately molecular weight is 70 kDa.

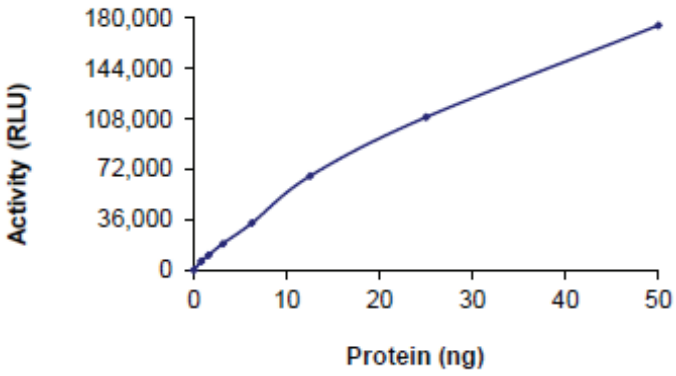

Figure 2: The specific activity of COT was determined to be 21 nmol/min/mg as per Activity Assay Protocol and was equivalent to 1164 nmol/min/mg as per radiometric assay.

REFERENCES

1. Channavajhala, P.L. *et al.* (2003) J. Biol. Chem. **278**:47089.
2. Rodriguez, C. *et al.* (2006) Cell Signal. **18**:1376.

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ACTIVITY ASSAY PROTOCOL

Solutions Required

Active Kinase - Active COT (0.1 µg/µL) diluted with Kinase Dilution Buffer IX (1X) and assayed as outlined in Figure 2.
Note: *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*

Kinase Assay Buffer III (5X) - 200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, and 0.5 mg/mL BSA. Add fresh DTT prior to use to a final concentration of 250 µM.

Kinase Dilution Buffer IX (1X) - Kinase Assay Buffer III diluted at a 1:4 ratio (5X dilution) with cold distilled water. Add fresh DTT prior to use to a final concentration of 50 µM.

ADP-Glo™ Kinase Assay Kit - 10 mM ATP Solution, 10 mM ADP Solution, ADP-Glo™ Reagent, Kinase Detection Reagent.

Substrate - Unactive MEK1 diluted in 50 mM sodium phosphate, pH 7.0, 300 mM NaCl, 150 mM imidazole, 0.1 mM PMSF, 2 mM DTT, 25% glycerol to a final concentration of 0.2 mg/mL.

ASSAY PROCEDURE

The COT assay is performed using the ADP-Glo™ Kinase Assay Kit which quantifies the amount of ADP produced by the COT reaction. The ADP-Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

1. Thaw the Active COT, Kinase Assay Buffer III (5X), and Substrate on ice. Prepare a 15 µL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1X), in a pre-chilled 96-well plate.
2. Prepare a substrate/ATP mixture as follows (25 µM example):

Reaction Component	Amount
10 mM ATP Solution	1 µL
Kinase Assay Buffer III (5x)	79 µL
Substrate at 0.5 mg/mL	80 µL

3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5 µL:

Component 1	3 µL of diluted Active COT.
Component 2	2 µL of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1X).
5. Incubate at ambient temperature for 40 minutes.
6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5 µL of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.
7. Add 10 µL of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader.
9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)
From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)
Corrected RLU from reaction / [(SA of ADP in RLU/pmol) * (Reaction time in min) * (Enzyme amount in µg or mg)]