Recombinant Human Active COT

Certificate of Analysis

Catalog Number: 4586-KS
Lot Number: 1478415

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.
Molecular Mass	♦ The approximate molecular weight is 70 kDa (see Figure 1 below).
Purity	♦ The purity was determined to be > 90% by densitometry (see Figure 1 below).
Formulation	♦ Supplied in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.
Size	♦ 10 μg.
Concentration	♦ 0.1 μg/μL.
Activity	♦ The specific activity of COT was determined to be 1150 nmol/min/mg using MEK1 and ERK1 substrates and a myelin basic protein (MBP) substrate (see Activity Assay Protocol).
Storage	♦ This product is stable at ≤ -70° C for up to 1 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.**

COT

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/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-1β mRNA levels (2).

References

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

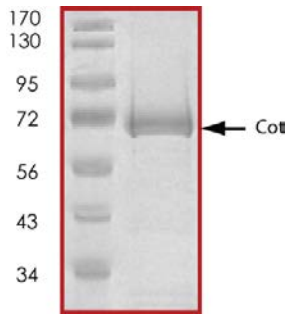

Figure 1: The approximate molecular weight is 70 kDa and the purity is > 90%.

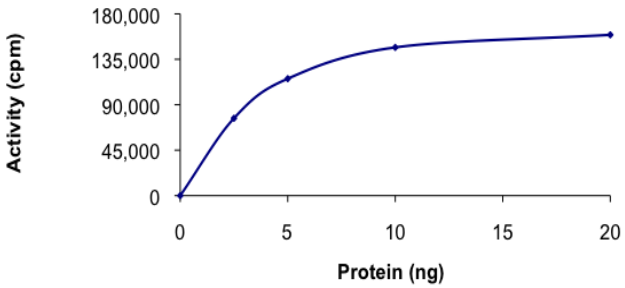

Figure 2: The specific activity of this lot of COT was determined to be 1150 nmol/min/mg as per the Activity Assay Protocol (on reverse).

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Activity Assay Protocol

Solutions Required

- **Active Kinase** - Active COT (0.1 μg/μL) diluted with Kinase Dilution Buffer. **Note:** *These are suggested working dilutions. Optimal dilutions should be determined by each laboratory for each application.*
- **Kinase Assay Buffer I, pH 7.2** - 25 mM MOPS, 12.5 mM β-glycerolphosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA. Add 0.25 mM DTT to the Kinase Assay Buffer prior to use.
- **Kinase Dilution Buffer, pH 7.2** - Kinase Assay Buffer I diluted 5-fold with a 50 ng/μL BSA solution.
- **10 mM ATP Stock Solution** - Prepare the ATP Stock Solution by dissolving 55 mg of ATP in 10 mL of Kinase Assay Buffer I. Store 200 μL aliquots at ≤ -20° C.
- **[^{33}P]-ATP Assay Cocktail** - Prepare 250 μM [^{33}P]-ATP Assay Cocktail in a designated radioactive work area by combining 150 μL of 10 mM ATP Stock Solution, 100 μL of [^{33}P]-ATP (1 mCi/100 μL), and 5.75 mL of Kinase Assay Buffer I. Store 1 mL aliquots at ≤ -20° C.
- **Substrate** - Inactive MEK1 and ERK1 were activated in a coupled reaction. Myelin Basic Protein (MBP) diluted in distilled or deionized water to a final concentration of 1 mg/mL was subsequently used as a substrate for the activated ERK1.

Assay Procedure

1. Thaw the Active COT, Kinase Assay Buffer I, and inactive MEK1 and ERK1 on ice. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20 μL.

Reaction Component	Amount
Diluted Active COT	10 μL
Inactive MEK1 (0.2 μg/μL)	2 μL
Inactive ERK1 (0.2 μg/μL)	3 μL
Kinase Dilution Buffer	5 μL

2. Start the reaction with the addition of 5 μL ATP (250 μM) and incubate in a water bath at 30° C for 25 minutes.
3. After the 25 minute incubation, remove 5 μL and add it to the following reaction components on ice, bringing the initial reaction volume up to 20 μL.

Reaction Component	Amount
Reaction Mixture	5 μL
Distilled or deionized water (on ice)	10 μL
MBP Substrate (1.0 mg/mL; on ice)	5 μL

4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled or deionized water.
5. Thaw the [^{33}P]-ATP Assay Cocktail in a shielded container in a designated radioactive work area. Initiate the reaction with the addition of 5 μL [^{33}P]-ATP Assay Cocktail, bringing the final volume up to 25 μL. Incubate the mixture in a water bath at 30° C for 15 minutes.
6. After the 15 minute incubation, terminate the reaction by spotting 20 μL of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (add 10 mL of phosphoric acid to 990 mL of distilled or deionized water) with constant gentle stirring. It is recommended that the strips be washed a total of three times for approximately 10 minutes each.
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 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)

Specific Activity (SA) = cpm for 5 μL [^{33}P]-ATP/pmole of ATP (in 5 μL of a 250 μM ATP stock solution, *i.e*. 1250 pmoles)

Calculation of Kinase Specific Activity (SA) (pmol/minutes/μg or nmol/minutes/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol) x (Reaction time in minutes) x (Enzyme amount in μg or mg)] x [(Reaction volume) / (Spot Volume)]

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