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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
M16-11G -05	5 µg
M16-11G -10	10 µg
M16-11G -20	20 µg

COT, Active

Recombinant human protein expressed in Sf9 cells

Catalog # M16-11G
Lot # I002-3

Product Description

Recombinant human COT (30-397) was expressed by baculovirus in Sf9 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

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

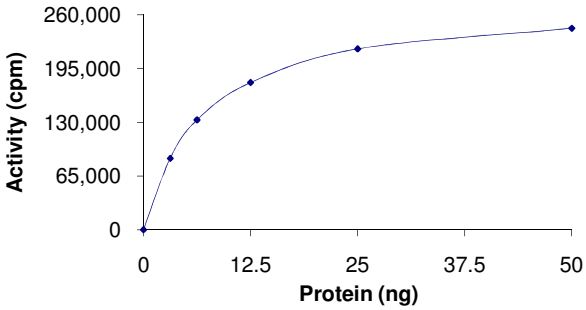
Scientific Background

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. C-terminal catalytic domain of KSR2 associates with COT and KSR2 can negatively regulates 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 IL8 production in a dose-dependent manner (1). 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-1betamRNA levels(2).

References

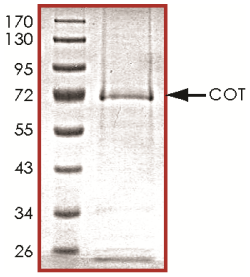
1. Channavajhala, P L. et al: Identification of a novel human kinase supporter of Ras (hKSR-2) that functions as a negative regulator of Cot (Tpl2) signaling. J. Biol. Chem. 278: 47089-47097, 2003.
2. Rodríguez C. et al: TRAF6 and Src kinase activity regulates Cot activation by IL-1. Cell Signal. 2006 Sep;18(9):1376-85.

Specific Activity



The specific activity of COT was determined to be **1100 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>75%** by densitometry.
Approx. MW **~70kDa**.

COT, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	M16-11G
Specific Activity	1100 nmol/min/mg
Specific Lot Number	I002-3
Purity	>75%
Concentration	0.1 µg/µl
Stability	1yr At –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: M16-11G)

Active COT (0.1µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active COT for optimal results).

Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^{33}P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

Substrate

Unactive MEK1 (Catalog #: M02-14G) and ERK1 (Catalog #: M29-14G) were activated in a coupled reaction. Myelin Basic Protein (MBP) (Catalog #: M42-51N) diluted in distilled H_2O to a final concentration of 1mg/ml was subsequently used as a substrate for the activated ERK1.

Assay Protocol

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

- **Component 1.** 10µl of diluted Active COT (Catalog #M16-11G)
- **Component 2.** 2µl of Unactive MEK1 (0.2µg/µl) (Catalog #M02-14G)
- **Component 3.** 3µl of Unactive ERK1 (0.2µg/µl) (Catalog #M29-14G)
- **Component 4.** 5µl of Kinase Dilution Buffer (Catalog #K23-09)

Step 2. Start the reaction by the addition of 5 µl ATP (250µM) and incubate in a water bath at 30°C for 25 minutes.

Step 3. After the 25 minute incubation period, remove 5µl and add to the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 5µl of reaction mixture
- **Component 2.** 10µl distilled H_2O on ice
- **Component 3.** 5µl of MBP substrate (1 mg/ml) on ice (Catalog #M42-51N)

Step 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 H_2O.

Step 5. Initiate the reaction by the addition of 5µl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

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

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

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

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

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