

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active COT [30 - 397]

<u>Enzyme description:-</u>	COT [30 – 397]
<u>Clone number:-</u>	DU 1810
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	2 mg/L
<u>Calculated molecular mass:-</u>	45, 146 daltons
<u>Purity:-</u>	75 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
	50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	
	Three step assay in which COT activates inactive MKK1 [DU 1843], which in turn activates inactive p42MAPKinase [DU 650 or DU 1844]. Activity of p42MAPKinase is then assayed against myelin basic protein as substrate (final concentration of 0.3 mg/ml), in the standard filter binding assay.
<u>Assay buffer:-</u>	
	50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.2 mM sodium vanadate, 0.5 µM microcystin-LR, 10 mM magnesium acetate
<u>Specific activity range:-</u>	To be determined

Division of Signal Transduction Therapy

Clone Data Sheet

COT [30 - 397]

<u>Protein</u>	COT [30 - 397]
<u>Clone Number</u>	DU 1810
<u>Species</u>	Human
<u>Accession number</u>	P41279
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	<pre>MSYYHHHHHHHDYDIPTTENLYFQGGAMGSMENLYASEEPAVYEPSLMTMC QDSNQNDERSKSLLLSGQEV PWLSSVRYGTVEDLLAFANHISNTAKH FY GQRPQESGILLNMVITPQNGRYQIDSDVLLIPWKLT YRNIGSDFIPRGA FGKVYLAQDIKTKKRMACKLIPVDQFKPSDVEIQACFRHENIAELYGAV LWGETVHLFMEAGEGGSVLEKLESCGPMREFEIIWVTKHVLKGLDFLHS KKVIHHDIKPSNIVFMSTKAVLVDFGLSVQMTEDVYFPKDLRGTEIYMS PEVILCRGHSTKADIYSLGATLIHMQTGTPPWVKRYPR SAYPSYLYIIH KQAPPLEDIADDCSPGMRELIEASLERNPNHRPRAADLLKHEALNPPRE DQPR</pre>
<u>Native sequence</u>	Amino acids M30 – R397 of human COT. [Full length native protein ends at residue G467] Residue M29 of fusion protein is equivalent to M30 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (<u>ENLYFOG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 site of pFastBAC HTb

Division of Signal Transduction Therapy

**Nucleotide
sequence of
insert**

GGATCCATGGAAAATCTTTATGCAAGTGAAGAGCCAGCAGTTTATGAAC
CCAGTCTAATGACCATGTGTCAAGACAGTAATCAAAACGATGAGCGTTC
TAAGTCTCTGCTGCTTAGTGGCCAAGAGGTACCATGGTTGTCATCAGTC
AGATATGGAACTGTGGAGGATTTGCTTGCTTTTGCAAACCATATATCCA
ACACTGCAAAGCATTTTTATGGACAACGACCACAGGAATCTGGAATTTT
ATTAACATGGTCATCACTCCCCAAAATGGACGTTACCAAATAGATTCC
GATGTTCTCCTGATCCCCTGGAAGCTGACTTACAGGAATATTGGTTCTG
ATTTTATTCTCGGGGCGCCTTTGGAAAGGTATACTTGGCTCAAGATAT
AAAGACGAAGAAAAGAATGGCGTGTAACCTGATCCCAGTAGATCAATTT
AAGCCATCTGATGTGGAATTCAGGCTTGCTTCCGGCACGAGAACATCG
CAGAGCTGTATGGCGCAGTCCTGTGGGGTGAAACTGTCCATCTCTTTAT
GGAAGCAGGCGAGGGAGGGTCTGTTCTGGAGAACTGGAGAGCTGTGGA
CCAATGAGAGAATTTGAAATTATTTGGGTGACAAAGCATGTTCTCAAGG
GACTTGATTTTCTACACTCAAAGAAAGTGATCCATCATGATATTAACC
TAGCAACATTGTTTTTCATGTCCACAAAAGCTGTTTTGGTGGATTTTGGC
CTAAGTGTTCAAATGACCGAAGATGTCTATTTTCCTAAGGACCTCCGAG
GAACAGAGATTTACATGAGCCCAGAGGTCATCCTGTGCAGGGGCCATTC
AACCAAAGCAGACATCTACAGCCTGGGGGCCACGCTCATCCACATGCAG
ACGGGCACCCACCCTGGGTGAAGCGCTACCCTCGCTCAGCCTATCCCT
CCTACCTGTACATAATCCACAAGCAAGCACCTCCACTGGAAGACATTGC
AGATGACTGCAGTCCAGGGATGAGAGAGCTGATAGAAGCTTCCCTGGAG
AGAAACCCCAATCACCGCCAAGAGCCGCAGACCTACTAAACATGAGG
CCCTGAACCCGCCAGAGAGGATCAGCCACGCTaagaattc