Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active TAO kinase 1 [1 – 356]

Enzyme description:- TAO kinase 1 [1 - 356]

Clone number:- DU 6956

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 67, 047.92 daltons
Average Mass 67, 090.89 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.6

Purity:- 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
Myelin Basic Protein Final concentration: 0.3 mg/ml

Specific activity range:- To be determined

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Clone Data Sheet

TAO kinase 1 [1 - 356]

Protein	TAO kinase 1 [1 - 356]
Clone number	DU 6956
Species	Human
Accession number	NM_020791
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MPSTNRAGSLKDPE** **IAELFFKEDPEKLFTDLREIGHGSFGAVYFARDVRTNEVVAIKKMSYSG** **KQSTEKWQDIIKEVKFLQRIKHPNSIEYKGCYLREHTAWLVMEYCLGSA** **SDLLEVHKKPLQEVEIAAITHGALQGLAYLHSHTMIHRDIKAGNILLTE** **PGQVKLADFGSASMASPANSFVGTPYWMAPEVILAMDEGQYDGKVDVWS** **LGITCIELAERKPPLFNMNAMSALYHIAQNESPTLQSNEWSDYFRNFVD** **SCLQKIPQDRPTSEELLKHIFVLRERPETVLIDLIQRTKDAVRELDNLQ** **YRKMKKLLFQEAHNGPAVEAQEEEEEQDHGVGRTGTVNSVGSNQSIPS**
Native sequence	Amino acids M1 – S356 (end T1001) of human TAO kinase 1. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 and *Not*1site of pGEX 6P-1

Nucleotide Sequence of insert

ggatccATGCCATCAACTAACAGAGCAGGCAGTCTTAAGGACCCTGAAA
TTGCAGAGCTCTTCTTCAAAGAAGATCCAGAGAAGCTCTTCACAGATCT
CAGAGAAATTGGCCATGGAAGCTTTGGAGCAGTGTATTTTGCACGAGAT
GTGCGTACCAATGAAGTGGTGGCCATCAAGAAAATGTCTTATAGTGGAA
AGCAGTCTACTGAGAAATGGCAGGATATTATTAAGGAAGTCAAGTTTCT
ACAAAGAATAAAACATCCCAACAGTATAGAATACAAAGGCTGTTATTTA
CGTGAACACACAGCATGGCTTGTAATGGAATATTGTTTAGGATCTGCTT
CGGATTTACTAGAAGTTCACAAAAAGCCATTACAAGAAGTGGAAATAGC
AGCAATTACACATGGTGCTCTTCAGGGATTAGCCTACTTACATTCTCAT
ACTATGATTCATAGAGATATCAAAGCAGGAAATATCCTTCTGACAGAAC
CAGGCCAGGTGAAACTTGCTGACTTTGGCTCTGCTTCCATGGCATCACC
TGCCAATTCCTTTGTGGGAACGCCGTATTGGATGGCCCCAGAAGTAATT
TTAGCCATGGATGAAGGACAATATGATGGCAAAGTAGATGTGTGGTCTC
TTGGAATAACATGTATTGAACTAGCGGAAAGGAAGCCTCCTTTATTTAA
TATGAATGCAATGAGTGCCTTATATCACATAGCCCAAAATGAATCCCCT
ACACTACAGTCTAATGAATGGTCTGATTATTTTCGCAACTTTGTAGATT
CTTGCCTCCAGAAAATCCCTCAAGATCGACCTACATCAGAGGAACTTTT
AAAGCACATATTTGTTCTTCGGGAGCGCCCTGAAACCGTGTTAATAGAT
CTCATTCAGAGGACAAAGGATGCAGTAAGAGAGCTGGACAATCTGCAGT
ATCGAAAGATGAAGAAACTCCTTTTCCAGGAGGCACATAATGGACCAGC
AGTAGAAGCACAGGAAGAAGAAGAGGAACAAGATCATGGTGTTGGCCGG
ACAGGAACAGTTAATAGTGTTGGAAGTAATCAATCCATTCCCAGCtagg
cggccgc