

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of TAOK2 [1 - 350]

<u>Enzyme description:-</u>	TAOK2 [1 - 350]
<u>Clone number:-</u>	DU 62949
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH-Sepharose

Calculated molecular mass:-

Monoisotopic 66, 325.61 daltons
Average Mass 66, 368.36 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.83

Purity:- >80 %

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 Buffer:-

50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-

Myelin Basic protein Final concentration: 1 mg/ml

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Clone Data Sheet

TAOK2 [1 - 350]

<u>Protein</u>	TAOK2 [1 - 350]
<u>Clone number</u>	DU 62949
<u>Species</u>	Human
<u>Accession number</u>	Q9UL54-1
<u>Tags</u>	N-terminal GST
<u>Baculovirus expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSM PAGGRAGSLKDPD VAELFFKDDPEKLFSDLREIGHGSFGAVYFARDVRNSEVVAIKMSYSG KQSNEKWQDI I KEVRFLQKLRHPNTIQYRGCYLREHTAWLVM EYCLGSA SDLLEVHKKPLQEVETAAVTHGALQGLAYLHSHNM IHRDVKAGNILLSE PGLVKLGDFGSASIMAPANSFVGT PYWMAPEVILAMDEGQYD GKVDVWS LGITCIELAERKPPLFNMNAMSALYHIAQNESPV LQSGHWSEYFRNFVD SCLQKIPQDRPTSEVLLKHRFVLRERPP TVIMDLIQRTKDAVRELDNLQ YRKMKKILFQEAPNGPGAEAPEEEEEAE P YMHRAGTLTSL ES</p>
<u>Native sequence</u>	Amino acids M1 – S350 (end residue is R1235) of human TAOK2. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
<u>Protease cleavage</u>	PreScission (LEVL FQGP) residues 221 – 228
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Not</i> 1 sites of pFastBac GST 6P1

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Nucleotide Sequence Of Insert

ggatccATGCCAGCTGGGGGCCGGGCCGGGAGCCTGAAGGACCCAGATG
TGGCTGAGCTCTTCTTCAAGGATGACCCAGAAAAGCTCTTCTCTGACCT
CCGGGAAATTGGCCATGGCAGCTTTGGAGCCGTATACTTTGCCCGGGAT
GTCCGGAATAGTGAGGTGGTGGCCATCAAGAAGATGTCCTACAGTGGGA
AGCAGTCCAATGAGAAATGGCAAGACATCATCAAGGAGGTGCGGTTCTT
ACAGAAGCTCCGGCATCCCAACACCATTTCAGTACCGGGGCTGTTACCTG
AGGGAGCACACGGCTTGGCTGGTAATGGAGTATTGCCTGGGCTCAGCTT
CTGACCTTCTAGAAGTGCACAAGAAACCCCTTCAGGAGGTAGAGATCGC
AGCTGTGACCCACGGGGCGCTTCAGGGCCTGGCATATCTGCACTCCCAC
AACATGATCCATAGGGATGTGAAGGCTGGAAACATCCTGCTGTCAGAGC
CAGGGTTAGTGAAGCTAGGGGACTTTGGTTCTGCGTCCATCATGGCACC
TGCCAACTCCTTCGTGGGCACCCCATACTGGATGGCACCCGAGGTGATC
CTGGCCATGGATGAGGGGCGAGTACGATGGCAAAGTGGACGTCTGGTCCT
TGGGGATAACCTGCATCGAGCTGGCTGAACGGAAACCACCGCTCTTTAA
CATGAATGCGATGAGTGCCTTATACCACATTGCACAGAACGAATCCCCC
GTGCTCCAGTCAGGACACTGGTCTGAGTACTTCCGGAATTTTGTGCGACT
CCTGTCTTCAGAAAATCCCTCAAGACAGACCAACCTCAGAGGTTCTCCT
GAAGCACCGCTTTGTGCTCCGGGAGCGGCCACCCACAGTCATCATGGAC
CTGATCCAGAGGACCAAGGATGCCGTGCGGGAGCTGGACAACCTGCAGT
ACCGCAAGATGAAGAAGATCCTGTTCCAAGAGGCACCCAACGGCCCTGG
TGCCGAGGCCCCAGAGGAGGAAGAGGAGCCGAGCCCTACATGCACCGG
GCCGGGACTCTGACCAGCCTCGAGAGTtgagcggccgc