Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active TTK [1 - 857]

Enzyme description:- TTK [1 – 857]

Clone number:- DU 8837

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 1 mg/L

Calculated molecular mass:-
Monoisotopic 100, 380.51 daltons
Average Mass 100, 443.89 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.10

Purity:- 75 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C [Long term stability to be determined]

Assay:- Standard filter binding assay

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

Substrate:-
RSRSRSRSRSRSRSR residues 204 – 218 of human ASF-1/SF-2
Final concentration: 300 μM

Specific activity range:- To be determined

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Clone Data Sheet

TTK [1 - 857]

Protein	TTK [1 – 857]
Clone Number	DU 8837
Species	Human
Accession number	NM_003318
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MESEDLSGRELTIDSIMNKVR DIKNKFKNEDLTDELSLNKISADTTDNSGTVNQIMMMANNPEDWLSLLL KLEKNSVPLSDALLNKLIGRYSQAIEALPPDKYGQNESFARIQVRFAEL KAIQEPDDARDYFQMARANCKKFAFVHISFAQFELSQGNVKKSKQLLQK AVERGAVPLEMLEIALRNLNLQKKQLLSEEEKKNLSASTVLTAQESFSG SLGHLQNRNNSCDSRGQTTKARFLYGENMPPQDAEIGYRNSLRQTNKTK QSCPFGRVPVNLLNSPDCDVKTDDSVVPCFMKRQTSRSECRDLVVPGSK PSGNDSCELRNLKSVQNSHFKEPLVSDEKSSELIITDSITLKNKTESSL LAKLEETKEYQEPEVPESNQKQWQSKRKSECINQNPAASSNHWQIPELA RKVNTEQKHTTFEQPVFSVSKQSPPISTSKWFDPKSICKTPSSNTLDDY MSCFRTPVVKNDFPPACQLSTPYGQPACFQQQQHQILATPLQNLQVLAS SSANECISVKGRIYSILKQIGSGGSSKVFQVLNEKKQIYAIKYVNLEEA DNQTLDSYRNEIAYLNKLQQHSDKIIRLYDYEITDQYIYMVMECGNIDL NSWLKKKKSIDPWERKSYWKNMLEAVHTIHQHGIVHSDLKPANFLIVDG MLKLIDFGIANQMQPDTTSVVKDSQVGTVNYMPPEAIKDMSSSRENGKS KSKISPKSDVWSLGCILYYMTYGKTPFQQIINQISKLHAIIDPNHEIEF PDIPEKDLQDVLKCCLKRDPKQRISIPELLAHPYVQIQTHPVNQMAKGT TEEMKYVLGQLVGLNSPNSILKAAKTLYEHYSGGESHNSSSSKTFEKKR GKK**
Native sequence	Amino acids M1 – K857 (end) of human TTK. Residue M29 of fusion protein is equilivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 and *Not*1 site of pFastBAC HTb

Nucleotide sequence of insert

ggatccATGGAATCCGAGGATTTAAGTGGCAGAGAATTGACAATTGATT
CCATAATGAACAAAGTGAGAGACATTAAAAATAAGTTTAAAAATGAAGA
CCTTACTGATGAACTAAGCTTGAATAAAATTTCTGCTGATACTACAGAT
AACTCGGGAACTGTTAACCAAATTATGATGATGGCAAACAACCCAGAGG
ACTGGTTGAGTTTGTTGCTCAAACTAGAGAAAAACAGTGTTCCGCTAAG
TGATGCTCTTTTAAATAAATTGATTGGTCGTTACAGTCAAGCAATTGAA
GCGCTTCCCCCAGATAAATATGGCCAAAATGAGAGTTTTGCTAGAATTC
AAGTGAGATTTGCTGAATTAAAAGCTATTCAAGAGCCAGATGATGCACG
TGACTACTTTCAAATGGCCAGAGCAAACTGCAAGAAATTTGCTTTTGTT
CATATATCTTTTGCACAATTTGAACTGTCACAAGGTAATGTCAAAAAAA
GTAAACAACTTCTTCAAAAAGCTGTAGAACGTGGAGCAGTACCACTAGA
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TTTCAGCAGATAATTAATCAGATTTCTAAATTACATGCCATAATTGATC
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TGTGTTAAAGTGTTGTTTAAAAAGGGACCCAAAACAGAGGATATCCATT
CCTGAGCTCCTGGCTCATCCCTATGTTCAAATTCAAACTCATCCAGTTA
ACCAAATGGCCAAGGGAACCACTGAAGAAATGAAATATGTTCTGGGCCA
ACTTGTTGGTCTGAATTCTCCTAACTCCATTTTGAAAGCTGCTAAAACT
TTATATGAACACTATAGTGGTGGTGAAAGTCATAATTCTTCATCCTCCA
AGACTTTTGAAAAAAAAAGGGGAAAAAAAtgagcggccgc