Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active IL2-inducible T-cell Kinase (ITK) [1 – 620]

Enzyme description:- ITK [1 – 620]

Clone number:- DU 35571

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 98, 591.72 daltons
Average Mass 98, 655.12 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.65

Purity:- 85 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.03 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- –70 °C

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

Substrate:-
Srctide [GEEPLYWSFPAKKK] Final concentration: 300 uM

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Clone Data Sheet

IL2-inducible T-cell Kinase (ITK) [1 – 620]

Protein	ITK [1 - 620]
Clone number	DU 35571
Species	Human
Accession number	NM_005546
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGS**MNNFILLEEQLIKKSQQKRRTSPSNFKVRFFVLTKASLA** **YFEDRHGKKRTLKGSIELSRIKCVEIVKSDISIPCHYKYPFQVVH** **DNYLLYVFAPDRESRQRWVLALKEETRNNNSLVPKYHPNFWMDGK** **WRCCSQLEKLATGCAQYDPTKNASKKPLPPTPEDNRRPLWEPEET** **VVIALYDYQTNDPQELALRRNEEYCLLDSSEIHWWRVQDRNGHEG** **YVPSSYLVEKSPNNLETYEWYNKSISRDKAEKLLLDTGKEGAFMV** **RDSRTAGTYTVSVFTKAVVSENNPCIKHYHIKETNDNPKRYYVAE** **KYVFDSIPLLINYHQHNGGGLVTRLRYPVCFGRQKAPVTAGLRYG** **KWVIDPSELTFVQEIGSGQFGLVHLGYWLNKDKVAIKTIREGAMS** **EEDFIEEAEVMMKLSHPKLVQLYGVCLEQAPICLVFEFMEHGCLS** **DYLRTQRGLFAAETLLGMCLDVCEGMAYLEEACVIHRDLAARNCL** **VGENQVIKVSDFGMTRFVLDDQYTSSTGTKFPVKWASPEVFSFSR** **YSSKSDVWSFGVLMWEVFSEGKIPYENRSNSEVVEDISTGFRLYK** **PRLASTHVYQIMNHCWKERPEDRPAFSRLLRQLAEIAESGL**
Native sequence	Amino acids M1 – L620 of human ITK. 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 (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Not*1sites of pFB DUAL6P-1.

Nucleotide sequence of insert

ggatccATGAACAACTTTATCCTCCTGGAAGAACAGCTCATCAAG
AAATCCCAACAAAAGAGAAGAACTTCTCCCTCGAACTTTAAAGTC
CGCTTCTTTGTGTTAACCAAAGCCAGCCTGGCATACTTTGAAGAT
CGTCATGGGAAGAAGCGCACGCTGAAGGGGTCCATTGAGCTCTCC
CGAATCAAATGTGTTGAGATTGTGAAAAGTGACATCAGCATCCCA
TGCCACTATAAATACCCGTTTCAGGTGGTGCATGACAACTACCTC
CTATATGTGTTTGCTCCAGATCGTGAGAGCCGGCAGCGCTGGGTG
CTGGCCCTTAAAGAAGAAACGAGGAATAATAACAGTTTGGTGCCT
AAATATCATCCTAATTTCTGGATGGATGGGAAGTGGAGGTGCTGT
TCTCAGCTGGAGAAGCTTGCAACAGGCTGTGCCCAATATGATCCA
ACCAAGAATGCTTCAAAGAAGCCTCTTCCTCCTACTCCTGAAGAC
AACAGGCGACCACTTTGGGAACCTGAAGAAACTGTGGTCATTGCC
TTATATGACTACCAAACCAATGATCCTCAGGAACTCGCACTGCGG
CGCAACGAAGAGTACTGCCTGCTGGACAGTTCTGAGATTCACTGG
TGGAGAGTCCAGGACAGGAATGGGCATGAAGGATATGTACCAAGC
AGTTATCTGGTGGAAAAATCTCCAAATAATCTGGAAACCTATGAG
TGGTACAATAAGAGTATCAGCCGAGACAAAGCTGAAAAACTTCTT
TTGGACACAGGCAAAGAAGGAGCCTTCATGGTAAGGGATTCCAGG
ACTGCAGGAACATACACCGTGTCTGTTTTCACCAAGGCTGTTGTA
AGTGAGAACAATCCCTGTATAAAGCATTATCACATCAAGGAAACA
AATGACAATCCTAAGCGATACTATGTGGCTGAAAAGTATGTGTTC
GATTCCATCCCTCTTCTCATCAACTATCACCAACATAATGGAGGA
GGCCTGGTGACTCGACTCCGGTATCCAGTTTGTTTTGGGAGGCAG
AAAGCCCCAGTTACAGCAGGGCTGAGATACGGGAAATGGGTGATC
GACCCCTCAGAGCTCACTTTTGTGCAAGAGATTGGCAGTGGGCAA
TTTGGGTTGGTGCATCTGGGCTACTGGCTCAACAAGGACAAGGTG
GCTATCAAAACCATTCGGGAAGGGGCTATGTCAGAAGAGGACTTC
ATAGAGGAGGCTGAAGTAATGATGAAACTCTCTCATCCCAAACTG
GTGCAGCTGTATGGGGTGTGCCTGGAGCAGGCCCCCATCTGCCTG
GTGTTTGAGTTCATGGAGCACGGCTGCCTGTCAGATTATCTACGC
ACCCAGCGGGGACTTTTTGCTGCAGAGACCCTGCTGGGCATGTGT
CTGGATGTGTGTGAGGGCATGGCCTACCTGGAAGAGGCATGTGTC
ATCCACAGAGACTTGGCTGCCAGAAATTGTTTGGTGGGAGAAAAC
CAAGTCATCAAGGTGTCTGACTTTGGGATGACAAGGTTCGTTCTG
GATGATCAGTACACCAGTTCCACAGGCACCAAATTCCCGGTGAAG
TGGGCATCCCCAGAGGTTTTCTCTTTCAGTCGCTATAGCAGCAAG
TCCGATGTGTGGTCATTTGGTGTGCTGATGTGGGAAGTTTTCAGT
GAAGGCAAAATCCCGTATGAAAACCGAAGCAACTCAGAGGTGGTG
GAAGACATCAGTACCGGATTTCGGTTGTACAAGCCCCGGCTGGCC
TCCACACACGTCTACCAGATTATGAATCACTGCTGGAAAGAGAGA
CCAGAAGATCGGCCAGCCTTCTCCAGACTGCTGCGTCAACTGGCT
GAAATTGCAGAATCAGGACTTtaggaattcgcaagggcgaattct
gcagatatccatcacactggcggccg