University of Dundee

Standard Operating Procedure

Preparation of active Spleen Tyrosine Kinase (SYK) [1 - 635]

Enzyme description:- SYK [1 - 635]

Clone number:- DU 8829

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic 75, 389.70 daltons
Average Mass 75, 437.83 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.92

Purity:- >80 %

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, 0.2 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, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:- Poly Glu Tyr (4:1) Final concentration: 1 mg/ml

Specific activity range:- To be determined

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Clone Data Sheet

SYK [1- 635]

Protein	SYK [1 - 635]
Clone number	DU 8829
Species	Human
Accession number	AAH01645.1
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MASSGMADSANHLPFFFGNIT REEAEDYLVQGGMSDGLYLLRQSRNYLGGFALSVAHGRKAHHYTIEREL NGTYAIAGGRTHASPADLCHYHSQESDGLVCLLKKPFNRPQGVQPKTGP FEDLKENLIREYVKQTWNLQGQALEQAIISQKPQLEKLIATTAHEKMPW FHGKISREESEQIVLIGSKTNGKFLIRARDNNGSYALCLLHEGKVLHYR IDKDKTGKLSIPEGKKFDTLWQLVEHYSYKADGLLRVLTVPCQKIGTQG NVNFGGRPQLPGSHPATWSAGGIISRIKSYSFPKPGHRKSSPAQGNRQE STVSFNPYEPELAPWAADKGPQREALPMDTEVYESPYADPEEIRPKEVY LDRKLLTLEDKELGSGNFGTVKKGYYQMKKVVKTVAVKILKNEANDPAL KDELLAEANVMQQLDNPYIVRMIGICEAESWMLVMEMAELGPLNKYLQQ NRHVKDKNIIELVHQVSMGMKYLEESNFVHRDLAARNVLLVTQHYAKIS DFGLSKALRADENYYKAQTHGKWPVKWYAPECINYYKFSSKSDVWSFGV LMWEAFSYGQKPYRGMKGSEVTAMLEKGERMGCPAGCPREMYDLMNLCW TYDVENRPGFAAVELRLRNYYYDVVN**
Native sequence	Amino acids M1 – N635 (end) of human SYK. Residue M29 of the fusion protein is equivalent 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 sites of pFastBAC HTb

Nucleotide Sequence of insert

ggatccATGGCCAGCAGCGGCATGGCTGACAGCGCCAACCACCTGCCCT
TCTTTTTCGGCAACATCACCCGGGAGGAGGCAGAAGATTACCTGGTCCA
GGGGGGCATGAGTGATGGGCTTTATTTGCTGCGCCAGAGCCGCAACTAC
CTGGGTGGCTTCGCCCTGTCCGTGGCCCACGGGAGGAAGGCACACCACT
ACACCATCGAGCGGGAGCTGAATGGCACCTACGCCATCGCCGGTGGCAG
GACCCATGCCAGCCCCGCCGACCTCTGCCACTACCACTCCCAGGAGTCT
GATGGCCTGGTCTGCCTCCTCAAGAAGCCCTTCAACCGGCCCCAAGGGG
TGCAGCCCAAGACTGGGCCCTTTGAGGATTTGAAGGAAAACCTCATCAG
GGAATATGTGAAGCAGACATGGAACCTGCAGGGTCAGGCTCTGGAGCAG
GCCATCATCAGTCAGAAGCCTCAGCTGGAGAAGCTGATCGCTACCACAG
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CATCCCCGAGGGAAAGAAGTTCGACACGCTCTGGCAGCTAGTCGAGCAT
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CCCAAGGGAACCGGCAAGAGAGTACTGTGTCATTCAATCCGTATGAGCC
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CCCATGGACACAGAGGTGTACGAGAGCCCCTACGCGGACCCTGAGGAGA
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GAAGCCATATCGAGGGATGAAAGGAAGTGAAGTCACCGCTATGTTAGAG
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CGCAGCAGTGGAACTGCGGCTGCGCAATTACTACTATGACGTGGTGAAC
taagcggccgc