Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active LYN [1 - 512]

Enzyme description:- LYN [1 – 512]

Clone number:- DU 51732

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 85, 343.43 daltons
Average Mass 85, 398.25 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.21

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 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, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
GGMEDIYEFMGGKKK Final concentration: 300 uM

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Clone Data Sheet

LYN [1 - 512]

Protein	LYN [1 - 512]
Clone number	DU 51732
Species	Human
Accession number	P07948.3
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MGCIKSKGK** **DSLSDDGVDLKTQPVRNTERTIYVRDPTSNKQQRPVPESQLLPGQRFQ** **TKDPEEQGDIVVALYPYDGIHPDDLSFKKGEKMKVLEEHGEWWKAKSL** **LTKKEGFIPSNYVAKLNTLETEEWFFKDITRKDAERQLLAPGNSAGAF** **LIRESETLKGSFSLSVRDFDPVHGDVIKHYKIRSLDNGGYYISPRITF** **PCISDMIKHYQKQADGLCRRLEKACISPKPQKPWDKDAWEIPRESIKL** **VKRLGAGQFGEVWMGYYNNSTKVAVKTLKPGTMSVQAFLEEANLMKTL** **QHDKLVRLYAVVTREEPIYIITEYMAKGSLLDFLKSDEGGKVLLPKLI** **DFSAQIAEGMAYIERKNYIHRDLRAANVLVSESLMCKIADFGLARVIE** **DNEYTAREGAKFPIKWTAPEAINFGCFTIKSDVWSFGILLYEIVTYGK** **IPYPGRTNADVMTALSQGYRMPRVENCPDELYDIMKMCWKEKAEERPT** **FDYLQSVLDDFYTATEGQYQQQP**
Native sequence	Amino acids M1 – P512 (end) of human LYN. 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 site (LEVLFQGP) residues 221 – 228
Cloning sites	*Bam*H1 and *Not*1 sites of pFastBac GST 6P1

Nucleotide sequence of insert

ggatccATGGGATGTATAAAATCAAAAGGGAAAGACAGCTTGAGTGAC
GATGGAGTAGATTTGAAGACTCAACCAGTACGTAATACTGAAAGAACT
ATTTATGTGAGAGATCCAACGTCCAATAAACAGCAAAGGCCAGTTCCA
GAATCTCAGCTTTTACCTGGACAGAGGTTTCAAACTAAAGATCCAGAG
GAACAAGGAGACATTGTGGTAGCCTTGTACCCCTATGATGGCATCCAC
CCGGACGACTTGTCTTTCAAGAAAGGAGAGAAGATGAAAGTCCTGGAG
GAGCATGGAGAATGGTGGAAAGCAAAGTCCCTTTTAACAAAAAAAGAA
GGCTTCATCCCCAGCAACTATGTGGCCAAACTCAACACCTTAGAAACA
GAAGAGTGGTTTTTCAAGGATATAACCAGGAAGGACGCAGAAAGGCAG
CTTTTGGCACCAGGAAATAGCGCTGGAGCTTTCCTTATTAGAGAAAGT
GAAACATTAAAAGGAAGCTTCTCTCTGTCTGTCAGAGACTTTGACCCT
GTGCATGGTGATGTTATTAAGCACTACAAAATTAGAAGTCTGGATAAT
GGGGGCTATTACATCTCTCCACGAATCACTTTTCCCTGTATCAGCGAC
ATGATTAAACATTACCAAAAGCAGGCAGATGGCTTGTGCAGAAGATTG
GAGAAGGCTTGTATTAGTCCCAAGCCACAGAAGCCATGGGATAAAGAT
GCCTGGGAGATCCCCCGGGAGTCCATCAAGTTGGTGAAAAGGCTTGGC
GCTGGGCAGTTTGGGGAAGTCTGGATGGGTTACTATAACAACAGTACC
AAGGTGGCTGTGAAAACCCTGAAGCCAGGAACTATGTCTGTGCAAGCC
TTCCTGGAAGAAGCCAACCTCATGAAGACCCTGCAGCATGACAAGCTC
GTGAGGCTCTACGCTGTGGTCACCAGGGAGGAGCCCATTTACATCATC
ACCGAGTACATGGCCAAGGGCAGTTTGCTGGATTTCCTGAAGAGCGAT
GAAGGTGGCAAAGTGCTGCTTCCAAAGCTCATTGACTTTTCTGCTCAG
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GACCTGCGAGCAGCTAATGTTCTGGTCTCCGAGTCACTCATGTGCAAA
ATTGCAGATTTTGGCCTTGCTAGAGTAATTGAAGATAATGAGTACACA
GCAAGGGAAGGTGCTAAGTTCCCTATTAAGTGGACGGCTCCAGAAGCA
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AGAACTAATGCCGACGTGATGACCGCCCTGTCCCAGGGCTACAGGATG
CCCCGTGTGGAGAACTGCCCAGATGAGCTCTATGACATTATGAAAATG
TGCTGGAAAGAAAAGGCAGAAGAGAGACCAACGTTTGACTACTTACAG
AGCGTCCTGGATGATTTCTACACAGCCACGGAAGGGCAATACCAGCAG
CAGCCTtaggcggccgc