Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ LYN

LYN proto-oncogene, Src family tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: LYN

Synonyms: JTK8

Product No.: 0358-0000-1

Lot: 001

Description: Human LYN, full length, amino acids M_1-P_{512} (as in NCBI/Protein entry NP_002341.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: LYN Lot 001, was confirmed as LYN by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 88,470 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

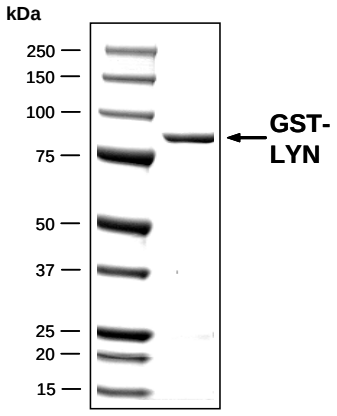
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.222 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

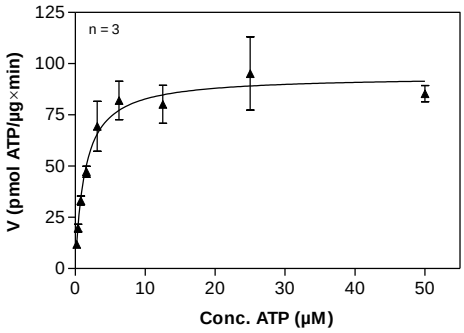
Biochemical Parameters:
Specific kinase activity (P_i transfer): 94 pmol/µg × min
ATP-K_M: 1.4 µM

LYN Lot 001:
Coomassie stain



2.0 µg GST-LYN

LYN Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
• Assay conditions:
60 mM HEPES-NaOH, pH 7.5
3 mM $MgCl_2$
3 mM $MnCl_2$
3 µM Na-orthovanadate
1.2 mM DTT
50 µg/ml $PEG_{20.000}$
ATP (variable)
Substrate: Poly(Glu:Tyr)$_{4:1}$, 40 µg/ml
LYN: 1 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

Additional assay technology:
LYN Lot 001 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

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GST-LYN Recombinant Fusion Protein Amino Acid Sequence

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   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
 241 LVPRGSPGLD GIYARGIQAS MGCIKSKGKD SLSDDGVDLK TQPVRNTERT IYVRDPTSNK 300
 301 QQRPVPESQL LPGQRFQTKD PEEQGDIVVA LYPYDGIHPD DLSFKKGEKM KVLEEHGEWW 360
 361 KAKSLLTKKE GFIPSNYVAK LNTLETEEWF FKDITRKDAE RQLLAPGNSA GAFLIRESET 420
 421 LKGSFSLSVR DFDPVHGDVI KHYKIRSLDN GGYYISPRIT FPCISDMIKH YQKQADGLCR 480
 481 RLEKACISPK PQKPWDKDAW EIPRESIKLV KRLGAGQFGE VWMGYYNNST KVAVKTLKPG 540
 541 TMSVQAFLEE ANLMKTLQHD KLVRLYAVVT REEPIYIITE YMAKGSLLDF LKSDEGGKVL 600
 601 LPKLIDFSAQ IAEGMAYIER KNYIHRDLRA ANVLVSESLM CKIADFGLAR VIEDNEYTAR 660
 661 EGAKFPIKWT APEAINFGCF TIKSDVWSFG ILLYEIVTYG KIPYPGRTNA DVMTALSQGY 720
 721 RMPRVENCPD ELYDIMKMCW KEKAEERPTF DYLQSVLDDF YTATEGQYQQ QP        780
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: LYN

LYN wt[1] Amino Acid Sequence

```
   1 MGCIKSKGKD SLSDDGVDLK TQPVRNTERT IYVRDPTSNK QQRPVPESQL LPGQRFQTKD  60
  61 PEEQGDIVVA LYPYDGIHPD DLSFKKGEKM KVLEEHGEWW KAKSLLTKKE GFIPSNYVAK 120
 121 LNTLETEEWF FKDITRKDAE RQLLAPGNSA GAFLIRESET LKGSFSLSVR DFDPVHGDVI 180
 181 KHYKIRSLDN GGYYISPRIT FPCISDMIKH YQKQADGLCR RLEKACISPK PQKPWDKDAW 240
 241 EIPRESIKLV KRLGAGQFGE VWMGYYNNST KVAVKTLKPG TMSVQAFLEE ANLMKTLQHD 300
 301 KLVRLYAVVT REEPIYIITE YMAKGSLLDF LKSDEGGKVL LPKLIDFSAQ IAEGMAYIER 360
 361 KNYIHRDLRA ANVLVSESLM CKIADFGLAR VIEDNEYTAR EGAKFPIKWT APEAINFGCF 420
 421 TIKSDVWSFG ILLYEIVTYG KIPYPGRTNA DVMTALSQGY RMPRVENCPD ELYDIMKMCW 480
 481 KEKAEERPTF DYLQSVLDDF YTATEGQYQQ QP                              540
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blue: LYN sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002341.1

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