Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ FYN wt

FYN proto-oncogene, Src family tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: FYN

Synonyms: MGC45350, SLK, SYN, p59-Fyn

Product No.: 0352-0000-1

Lot: 002

Description: Human FYN (Isoform A), full length, amino acids M_1-L_{537} (as in NCBI/Protein entry NP_002028.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: FYN wt Lot 002, was confirmed as FYN by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 90,358 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

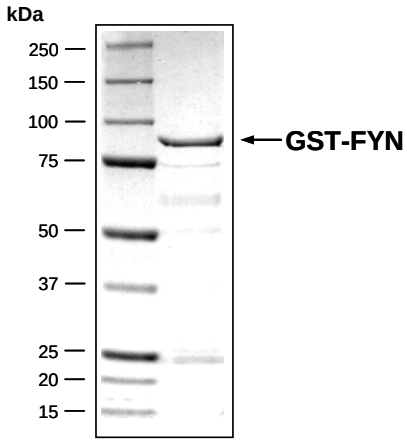
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

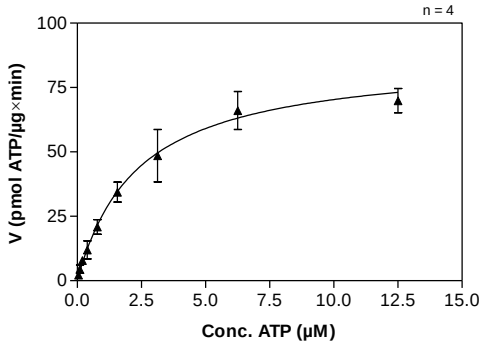
Biochemical Parameters:
Specific kinase activity (P_i transfer): 87 pmol/µg × min
ATP-K_M: 2.4 µM

FYN wt Lot 002:
Coomassie stain



2.0 µg GST-FYN

FYN wt Lot 002:
Determination of V_{max} and K_M value for ATP



- 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: $Poly(Glu:Tyr)_{4:1}$, 20 µg/ml
  Kinase: 2 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
FYN wt Lot 002 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

ProQinase™ FYN wt

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GST-FYN wt 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 GICSRNSMGC VQCKDKEATK LTEERDGSLN QSSGYRYGTD PTPQHYPSFG 300
301 VTSIPNYNNF HAAGGQGLTV FGGVNSSSHT GTLRTRGGTG VTLFVALYDY EARTEDDLSF 360
361 HKGEKFQILN SSEGDWWEAR SLTTGETGYI PSNYVAPVDS IQAEEWYFGK LGRKDAERQL 420
421 LSFGNPRGTF LIRESETTKG AYSLSIRDWD DMKGDHVKHY KIRKLDNGGY YITTRAQFET 480
481 LQQLVQHYSE RAAGLCCRLV VPCHKGMPRL TDLSVKTKDV WEIPRESLQL IKRLGNGQFG 540
541 EVWMGTWNGN TKVAIKTLKP GTMSPESFLE EAQIMKKLKH DKLVQLYAVV SEEPIYIVTE 600
600 YMNKGSLLDF LKDGEGRALK LPNLVDMAAQ VAAGMAYIER MNYIHRDLRS ANILVGNGLI 660
661 CKIADFGLAR LIEDNEYTAR QGAKFPIKWT APEAALYGRF TIKSDVWSFG ILLTELVTKG 720
721 RVPYPGMNNR EVLEQVERGY RMPCPQDCPI SLHELMIHCW KKDPEERPTF EYLQSFLEDY 780
781 FTATEPQYQP GENL                                                  840
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: FYN

FYN wt[1] Amino Acid Sequence

```
  1 MGCVQCKDKE ATKLTEERDG SLNQSSGYRY GTDPTPQHYP SFGVTSIPNY NNFHAAGGQG  60
 61 LTVFGGVNSS SHTGTLRTRG GTGVTLFVAL YDYEARTEDD LSFHKGEKFQ ILNSSEGDWW 120
121 EARSLTTGET GYIPSNYVAP VDSIQAEEWY FGKLGRKDAE RQLLSFGNPR GTFLIRESET 180
181 TKGAYSLSIR DWDDMKGDHV KHYKIRKLDN GGYYITTRAQ FETLQQLVQH YSERAAGLCC 240
241 RLVVPCHKGM PRLTDLSVKT KDVWEIPRES LQLIKRLGNG QFGEVWMGTW NGNTKVAIKT 300
301 LKPGTMSPES FLEEAQIMKK LKHDKLVQLY AVVSEEPIYI VTEYMNKGSL LDFLKDGEGR 360
361 ALKLPNLVDM AAQVAAGMAY IERMNYIHRD LRSANILVGN GLICKIADFG LARLIEDNEY 420
421 TARQGAKFPI KWTAPEAALY GRFTIKSDVW SFGILLTELV TKGRVPYPGM NNREVLEQVE 480
481 RGYRMPCPQD CPISLHELMI HCWKKDPEER PTFEYLQSFL EDYFTATEPQ YQPGENL     540
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blue: kinase sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_002028.1

Recombinant Proteins