Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ AXL

AXL receptor tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: AXL

Synonyms: EC, UFO

Product No.: 0525-0000-1

Lot: 003

Description: Human AXL, C-terminal fragment, amino acids H_{464}-A_{885} (as in NCBI/Protein entry NP_001690.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: AXL, Lot 003, was confirmed as AXL by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 76,549 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

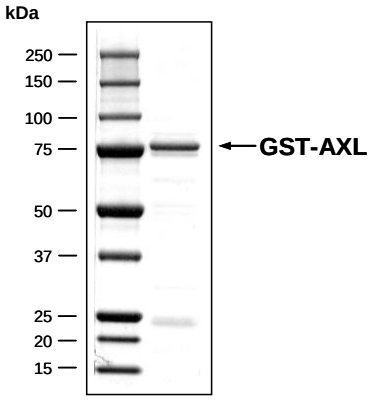
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

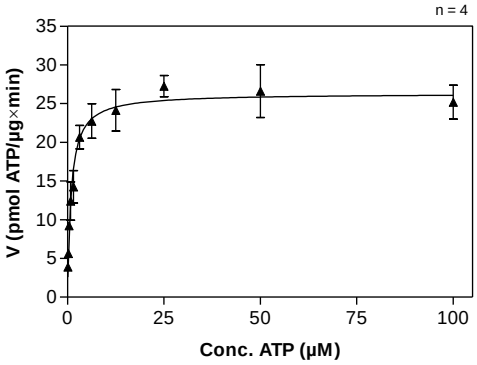
Biochemical Parameters:
Specific kinase activity (P_i transfer): 26 pmol/µg × min
ATP-K_M: 0.9 µM

AXL Lot 003:
Coomassie stain



2.0 µg GST-AXL

AXL Lot 003:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
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: $Poly(Glu:Tyr)_{4:1}$, 2.5 µg/ml
Kinase: 1.0 µg/ml
• Filter binding assay
MSFC membrane (Millipore)

Additional assay technology:
AXL Lot 003 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

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ AXL

Product No.: 0525-0000-1

GST-AXL Recombinant Fusion Protein Amino Acid Sequence

```
   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 GICSSHRRKK ETRYGEVFEP TVERGELVVR YRVRKSYSRR TTEATLNSLG  300
 301  ISEELKEKLR DVMVDRHKVA LGKTLGEGEF GAVMEGQLNQ DDSILKVAVK TMKIAICTRS  360
 361  ELEDFLSEAV CMKEFDHPNV MRLIGVCFQG SERESFPAPV VILPFMKHGD LHSFLLYSRL  420
 421  GDQPVYLPTQ MLVKFMADIA SGMEYLSTKR FIHRDLAARN CMLNENMSVC VADFGLSKKI  480
 481  YNGDYYRQGR IAKMPVKWIA IESLADRVYT SKSDVWSFGV TMWEIATRGQ TPYPGVENSE  540
 541  IYDYLRQGNR LKQPADCLDG LYALMSRCWE LNPQDRPSFT ELREDLENTL KALPPAQEPD  600
 600  EILYVNMDEG GGYPEPPGAA GGADPPTQPD PKDSCSCLTA AEVHPAGRYV LCPSTTPSPA  660
 661  QPADRGSPAA PGQEDGA                                                 720
```

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: AXL fragment

AXL wt[1] Amino Acid Sequence

```
   1  MAWRCPRMGR VPLAWCLALC GWACMAPRGT QAEESPFVGN PGNITGARGL TGTLRCQLQV   60
  61  QGEPPEVHWL RDGQILELAD STQTQVPLGE DEQDDWIVVS QLRITSLQLS DTGQYQCLVF  120
 121  LGHQTFVSQP GYVGLEGLPY FLEEPEDRTV AANTPFNLSC QAQGPPEPVD LLWLQDAVPL  180
 181  ATAPGHGPQR SLHVPGLNKT SSFSCEAHNA KGVTTSRTAT ITVLPQQPRN LHLVSRQPTE  240
 241  LEVAWTPGLS GIYPLTHCTL QAVLSDDGMG IQAGEPDPPE EPLTSQASVP PHQLRLGSLH  300
 301  PHTPYHIRVA CTSSQGPSSW THWLPVETPE GVPLGPPENI SATRNGSQAF VHWQEPRAPL  360
 361  QGTLLGYRLA YQGQDTPEVL MDIGLRQEVT LELQGDGSVS NLTVCVAAYT AAGDGPWSLP  420
 421  VPLEAWRPVK EPSTPAFSWP WWYVLLGAVV AAACVLILAL FLVHRRKKET RYGEVFEPTV  480
 481  ERGELVVRYR VRKSYSRRTT EATLNSLGIS EELKEKLRDV MVDRHKVALG KTLGEGEFGA  540
 541  VMEGQLNQDD SILKVAVKTM KIAICTRSEL EDFLSEAVCM KEFDHPNVMR LIGVCFQGSE  600
 600  RESFPAPVVI LPFMKHGDLH SFLLYSRLGD QPVYLPTQML VKFMADIASG MEYLSTKRFI  660
 661  HRDLAARNCM LNENMSVCVA DFGLSKKIYN GDYYRQGRIA KMPVKWIAIE SLADRVYTSK  720
 721  SDVWSFGVTM WEIATRGQTP YPGVENSEIY DYLRQGNRLK QPADCLDGLY ALMSRCWELN  780
 781  PQDRPSFTEL REDLENTLKA LPPAQEPDEI LYVNMDEGGG YPEPPGAAGG ADPPTQPDPK  840
 841  DSCSCLTAAE VHPAGRYVLC PSTTPSPAQP ADRGSPAAPG QEDGA                  900
```

blue: AXL sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_001690.2