

Certificate of Analysis

Axl, active
 (recombinant enzyme expressed in Sf21)
 Catalog # 14-512
 Lot # D8AN040AU

Product Description: N-terminal 5His-tagged, recombinant amino acids 473-end human Axl expressed in Sf21 cells. Purified using Ni²⁺/NTA agarose. Purity 66.2 % by SDS-PAGE and Coomassie blue staining. MW = 48 kDa.

Specific Activity (lot # D8AN040AU): 149U/mg, where one unit of Axl activity is defined as 1 nmol phosphate incorporated into 250 μM KKSREGDYMTMQIG per minute at 30 °C with a final ATP concentration of 100 μM.

Formulation: 1 mg of enzyme in 765.1 μl of 50 mM Tris/HCl pH 7.5, 150 mM NaCl, 0.1 mM EGTA, 0.03 % Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1 % 2-mercaptoethanol. Frozen solution.

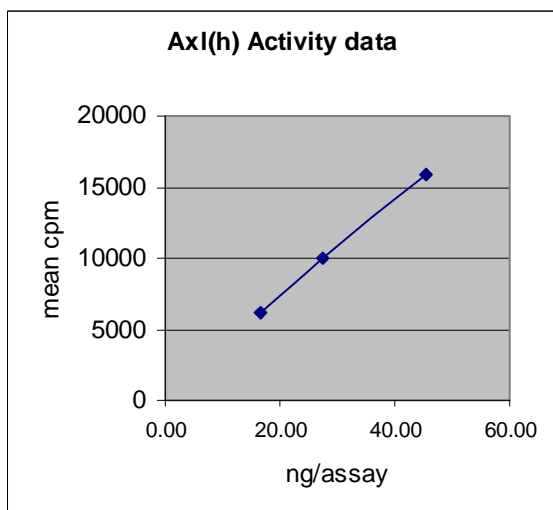
Storage and Stability: Store at -70 °C. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70 °C.

**FOR IN VITRO RESEARCH USE ONLY
 NOT FOR USE IN HUMANS OR ANIMALS**

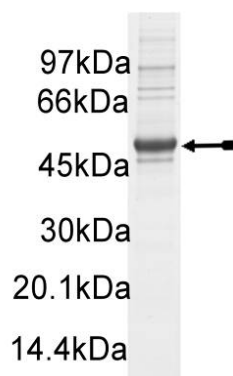
Quality Control Testing

Kinase Assay: 16 - 45ng of this lot of enzyme phosphorylated 250 μM KKSREGDYMTMQIG in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as Axl with 53% amino acid coverage of the translated sequence listed on page 3.

SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μg of Axl.



Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction buffer:** 40 mM MOPS/NaOH pH 7.0, 1 mM EDTA.
2. **KKSRGDYMTMQIG:** Use at a final assay concentration of 250 μ M. Prepare a 2.5 mM stock. Add 2.5 μ l of stock per assay point.
3. **Axl:** Dilute in 20 mM MOPS/NaOH pH 7.0, 1 mM EDTA, 0.01 % Brij-35, 5 % glycerol, 0.1 % 2-mercaptoethanol, 1 mg/ml BSA. Use 16 - 45 ng per assay point.
4. **$[\gamma\text{-}^{33}\text{P}]\text{ATP}$:** 2.5 x magnesium acetate/ $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ cocktail: 25 mM MgAc and 0.25 mM ATP to which is added $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ (specific activity approximately 500 - 800 cpm/pmol as required).

Assay Procedure (96 well plate assay format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of KKSRGDYMTMQIG.
3. Add **2.5 μ l (16 -45ng) Axl, active.**
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat.**
9. Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4 ml of scintillation cocktail.
12. Read in a scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain all enzyme components and 1 μ l 30 % phosphoric acid.

Axl Sequence Information

Protein human Axl

Tags N-terminal 5His tag.

Native sequence H7 of the recombinant protein is equivalent to H473 of human Axl.

Accession number GenBank NM_021913. The recombinant protein also contains the conflict Q764R with respect to GenBank NM_021913. This substitution is reported in GenBank BC032229. The residue coordinates in the native sequence are given.

Recombinant human Axl amino acid sequence:

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1 MHHHHHHRRK KETRYGEVFE PTVERGELVV RYRVRKSYSR RTTEATLNSL GISEELKEKL
61 RDVMVDRHKV ALGKTLGEGE FGAVMEGQLN QDDSILKVAV KTMKIAICTR SELEDFLSEA
121 VCMKEFDHPN VMRLIGVCFQ GSERESFPAP VVILPFMKHG DLHSFLLYSR LGDQPVYLP
181 QMLVKFMADI ASGMEYLSTK RFIHRDLAAR NCMLNENMSV CVADFGLSKK IYNGDYRQG
241 RIAKMPVKWI AIESLADRVY TSKSDVWSFG VTMWEIATRG QTPYPGVENS EIYDYLRRGN
301 RLKQPADCLD GLYALMSRCW ELNPQDRPSF TELREDLENT LKALPPAQEP DEILYVNMDE
361 GGGYPEPPGA AGGADPPTQP DPKDSCSCLT AAEVHPAGRY VLCPSTTPSP AQPADRGSPA
421 APGQEDGA
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Recombinant human Axl nucleotide sequence:

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1 atgcatcacc atcaccatca ccggcgaaag aaggagacc gttatggaga agtgtttgaa
61 ccaacagtgg aaagaggatga actggtagtc aggtaccgcg tgcgcaagtc ctacagtcgt
121 cggaccactg aagctacctt gaacagcttg ggcacagtg aagagctgaa ggagaagctg
181 cgggatgtga tgggtggaccg gcacaagggtg gccctgggga agactctggg agagggagag
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421 ggttctgaac gagagagctt cccagcacct gtggtcatct tacctttcat gaaacatgga
481 gacctacaca gcttcctcct ctatcccggg ctcggggacc agccagtgtg cctgccact
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1261 gccccagggc aggaggatgg tgcttga
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