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## Certificate of Analysis

### Axl, active

(Recombinant enzyme expressed in Sf21 insect cells.)

Catalogue # 14-512

Lot # 1621113

From bulk lot # D7SN042U

**Product Description:** N-terminal 5His-tagged, recombinant amino acids 473–end human Axl expressed in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Purity 84% by SDS-PAGE and Coomassie blue staining. MW = 48kDa.

**Specific Activity (lot# 1621113):** 142U/mg, where one unit of Axl activity is defined as 1nmol phosphate incorporated into 250µM (KKSRGDYMTMQIG) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 10µg of enzyme in 8.6µl of 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

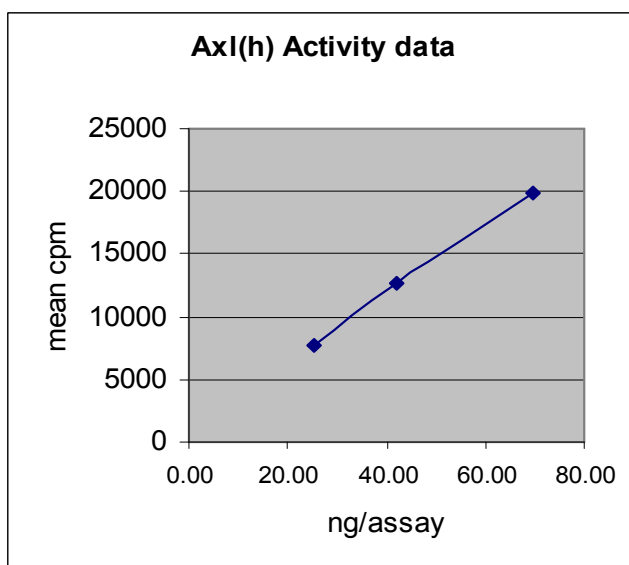
**Storage and Stability:** Store at -70°C. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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**

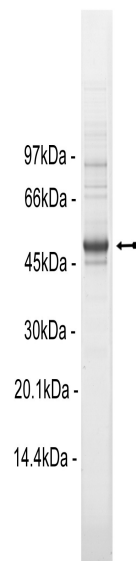
### Quality Control Testing

**Kinase Assay:** 25–70ng of this lot of enzyme phosphorylated 250µM (KKSRGDYMTMQIG) 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 42% amino acid coverage of the translated sequence listed on page three.

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



## Kinase Assay Protocol

### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKS<sub>R</sub>GDYMTMQIG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock. Add 2.5µl of stock per assay point.
3. **Axl, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 25–70ng per assay point.
4. **[γ-<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[γ-<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

### Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKS<sub>R</sub>GDYMTMQIG)**.
3. Add **2.5µl (25–70ng) Axl, active**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [γ-<sup>33</sup>P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all enzyme components plus 1µl of 30% phosphoric acid.

## Axl Sequence Information

<b>Protein</b>	human Axl
<b>Tags</b>	N-terminal 5His
<b>Native sequence</b>	H7 of the recombinant protein is equivalent to H473 of human Axl
<b>Accession number</b>	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:

```
1 MHHHHHRRK KETRYGEVFE PTVERGELVV RYRVRKSYSR RTTEATLNSL GISEELKEKL
61 RDVMVDRHKV ALGKTLGEGE FGAVMEGQLN QDDSIKLVAV KTMKIAICTR SELEDLSEA
121 VCMKEFDHPN VMRLIGVCFQ GSERESFPAP VVILPFMKHG DLHSFLLYSR LGDQPVYLPT
181 QMLVKFMADI ASGMEYLSLK RFIHRDLAAR NCMLNENMSV CVADFGLSKK IYNGDYRQG
241 RIAKMPVKWI AIESLADRVY TSKSDVWSFG VTMWEIATRG QTPYPGVENS EIYDYLRRGN
301 RLKQPADCLD GLYALMSRCV ELNPQDRPSF TELREDLENT LKALPPAQEP DEILYVNMDE
361 GGGYPEPPGA AGGADPPTQP DPKDSCSCLT AAEVHPAGRY VLCPSTTSPS AQPADRGSPA
421 APGQEDGA
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### Recombinant human Axl nucleotide sequence:

```
1 atgcatcacc atcaccatca cggcgaaaag aaggagaccc gttatggaga agtgtttgaa
61 ccaacagtgg aaagaggtga actggtagtc aggtaccgcg tgcgcaagtc ctacagtcgt
121 cggaccactg aagctacctt gaacagcttg ggcacagctg aagagctgaa ggagaagctg
181 cgggatgtga tgggtgaccg gcacaaggtg gccctgggga agactctggg agagggagag
241 tttggagctg tgatggaagg ccagctcaac caggacgact ccatcctcaa ggtggctgtg
301 aagacgatga agattgccat ctgcacgagg tcagagctgg aggatttctt gagtgaagcg
361 gtctgcatga aggaatttga ccatccaac gtcatgaggc tcatcgggtg ctgtttccag
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1141 gaccctaagg attcctgtag ctgcctcact gcggctgagg tccatcctgc tggacgctat
1201 gtcctctgcc ctccacaac ccctagcccc gctcagcctg ctgatagggg ctccccagca
1261 gccccagggc aggaggatgg tgcttga
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Reviewed and approved by site quality representative.

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