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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.

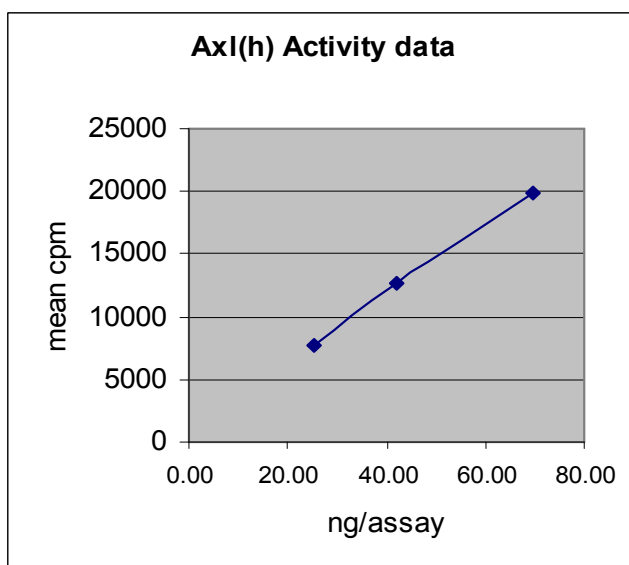
**Storage and Stability:** Store at -70°C. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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**

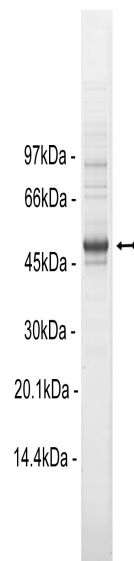
### Quality Control Testing

**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  MHHHHHHRK  KETRYGEVFE  PTVERGELVV  RYRVRKSYSR  RTTEATLNSL  GISEELKEKL
61  RDVMVDRHKV  ALGKTLGEGE  FGAVMEGQLN  QDDSIKLVAV  KTMKIAICTR  SELEDFLSEA
121  VCMKEFDHPN  VMRLIGVCFQ  GSERESFPAP  VVILPFMKHG  DLHSFLLYSR  LGDQPVYLPT
181  QMLVKFMADI  ASGMEYLSK  RFIHRDLAAR  NCMLNENMSV  CVADFGLSKK  IYNGDYRQG
241  RIAKMPVKWI  AIESLADRVY  TSKSDVWSFG  VTMWEIATRG  QTPYPGVENS  EIYDYLRRGN
301  RLKQPADCLD  GLYALMSRCV  ELNPQDRPSF  TELREDLENT  LKALPPAQEP  DEILYVNMDE
361  GGGYPEPPGA  AGGADPPTQP  DPKDSCSCLT  AAEVHPAGRY  VLCPSTTSP  AQPADRGSPA
421  APGQEDGA
```

### 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  tgggtggaccg  gcacaaggtg  gccctgggga  agactctggg  agagggagag
241  tttggagctg  tgatggaagg  ccagctcaac  caggacgact  ccatcctcaa  ggtggctgtg
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421  ggttctgaac  gagagagctt  cccagcacct  gtggtcatct  tacctttcat  gaaacatgga
481  gacctacaca  gtttctctct  ctattcccgg  ctcggggacc  agccagtgta  cctgcccact
541  cagatgctag  tgaagtctat  ggagacatc  gccagtgcca  tggagtatct  gagtaccaag
601  agattcatac  accgggacct  ggcgccagg  aactgcatgc  tgaatgagaa  catgtccgtg
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901  cgcctgaagc  agcctgcgga  ctgtctggat  ggactgtatg  ccttgatgct  gcggtgctgg
961  gagctaaatc  cccaggaccg  gccaaagttt  acagagctgc  gggaaagatt  ggagaacaca
1021  ctgaaggcct  tgcctcctgc  ccaggagcct  gacgaaatcc  tctatgtcaa  catggatgag
1081  ggtggagggt  atcctgaacc  ccctggagct  gcaggaggag  ctgaccccc  aaccagcca
1141  gaccctaagg  attcctgtag  ctgcctcact  gcggtgagg  tccatcctgc  tggacgctat
1201  gtcctctgcc  cttccacaac  ccctagcccc  gctcagcctg  ctgatagggg  ctccccagca
1261  gccccagggc  aggaggatgg  tgcttga
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Reviewed and approved by site quality representative.

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