

## Certificate of Analysis

### Axl, active

(Recombinant enzyme expressed in Sf21 insect cells.)

Item # 14-512, 14-512-K, 14-512M

Parent Lot # D7SN042U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**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 (Parent lot# D7SN042U):** 142U/mg, where one unit of Axl, active activity is defined as 1nmol phosphate incorporated into 250μM (KKSREGDYMTMQIG) per minute at 30°C with a final ATP concentration of 100μM.

**Formulation:** 1.158mg/ml of enzyme in 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.

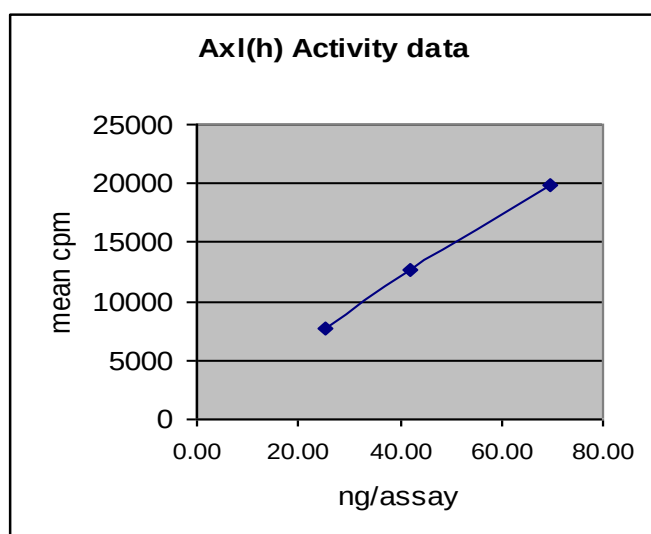
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge 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**

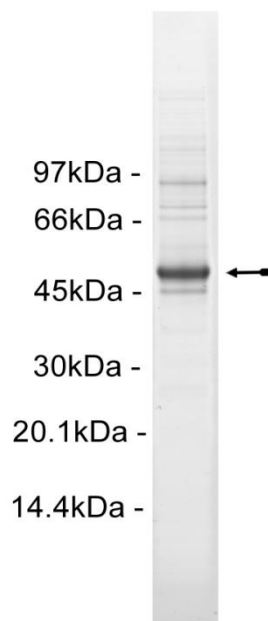
### Quality Control Testing

**Kinase Assay:** 25–70ng of this lot of enzyme phosphorylated 250μM (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 the translated sequence identified on page three.

**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3μg of Axl, active.



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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKSRGDYMTMQIG):** 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. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<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 **(KKSRGDYMTMQIG)**.
3. Add **2.5µl (25–70ng) Axl, active**.
4. Add 5µl of dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<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.

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## 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  KENTRYGEVFE  PTVERGELVV  RYRVRKSYSR  RTTEATLNSL  GISEELKEKL
61  RDVMVDRHKV  ALGKTLGEGE  FGAVMEGQLN  QDDSIKLVAV  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:

```

1  atgcatcacc  atccaccatca  ccggcgaaag  aaggagaccc  gttatggaga  agtgtttgaa
61  ccaacagtgg  aaagaggtga  actggtagtc  aggtaccgcg  tgcgcaagtc  ctacagtcgt
121 cggaccactg  aagctacctt  gaacagcttg  ggcatcagtg  aagagctgaa  ggagaagctg
181 cgggatgtga  tggtaggacc  gcacaaggtg  gccctgggga  agactctggg  agagggagag
241 tttggagctg  tggtaggaag  ccagctcaac  caggacgact  ccatcctcaa  ggtggctgtg
301 aagacagtga  agattgccat  ctgcacgagg  tcagagctgg  aggatttcct  gagtgaagcg
361 gtctgcatga  aggaatttga  ccatcccaac  gtcatgaggc  tcatcggtgt  ctgtttccag
421 ggttctgaac  gagagagctt  cccagcacct  gtggtcatct  tacctttcat  gaaacatgga
481 gacctacaca  gcttcctcct  ctattcccgg  ctcggggacc  agccagtgtg  cctgccact
541 cagatgctag  tgaagtctat  ggagacatc  gccagtggca  tggagtatct  gagtaccaag
601 agattcatac  accgggacct  ggcgggcagg  aactgcatgc  tgaatgagaa  catgtccgtg
661 tgtgtggcgg  acttcgggct  ctccaagaag  atctacaatg  gggactacta  ccgccaggga
721 cgtatcgcca  agatgccagt  caagtggatt  gccattgaga  gtctagctga  ccgtgtctac
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841 caaaccat  atccggcggt  ggagaacagc  gagatttatg  actatctgcg  ccggggaaat
901 cgctgaagc  agcctgcgga  ctgtctggat  ggactgtatg  ccttgatgtc  gcggtgctgg
961 gagctaaatc  cccaggaccg  gccaaagttt  acagagctgc  gggaagattt  ggagaacaca
1021 ctgaaggcct  tgcctcctgc  ccaggagcct  gacgaaatcc  tctatgtcaa  catggatgag
1081 ggtggagggt  atcctgaacc  ccctggagct  gcaggaggag  ctgaccccc  aaccagcca
1141 gaccctaagg  attcctgtag  ctgcctcact  gcggctgagg  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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