

## AXL, Active

Recombinant human protein expressed in Sf9 cells

**Catalog # A34-11H**

Lot # B1926-11

### Product Description

Recombinant human AXL (473-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is [NM\\_021913](#).

### Gene Aliases

UFO, JTK11

### Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.2mM DTT, 25% glycerol.

### Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

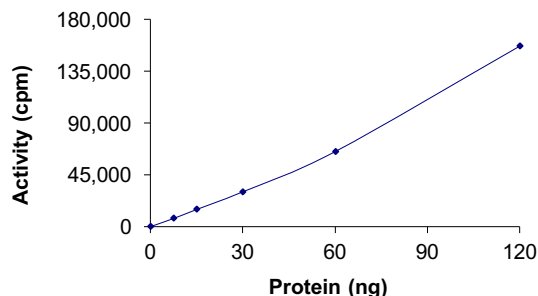
### Scientific Background

AXL is a member of the receptor tyrosine kinase family which has oncogenic potential and is implicated in human myeloid leukemia (1). AXL is a member of a complex signaling network that is involved in the control of cell proliferation and differentiation. Overexpression of AXL cDNA in NIH 3T3 cells induces neoplastic transformation of these cells with the concomitant appearance of a 140kDa AXL tyrosine-phosphorylated protein (2). Expression of AXL cDNA in the baculovirus system results in the expression of the appropriate recombinant protein that is recognized by antiphosphotyrosine antibodies, confirming that the AXL protein is tyrosine phosphorylated.

### References

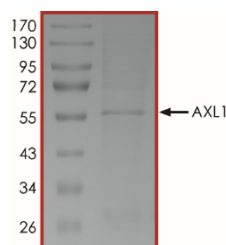
1. O'Bryan, J.P. et al: Axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. Mol Cell Biol. 1991 Oct;11(10):5016-31.
2. Janssen, J.W. et al: A novel putative tyrosine kinase receptor with oncogenic potential. Oncogene. 1991 Nov;6(11):2113-20.

### Specific Activity



The specific activity of AXL was determined to be **88 nmol /min/mg** as per activity assay protocol.

### Purity



The purity of AXL was determined to be **>70%** by densitometry, approx. MW **55kDa**.

## AXL, Active

Recombinant protein expressed in Sf9 cells

Catalog #	A34-11H
Specific Activity	88 nmol/min/mg
Lot #	B1926-11
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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# Activity Assay Protocol

## Reaction Components

### Active Kinase (Catalog #: A34-11H)

Active AXL (0.05µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active AXL for optimal results).

### Kinase Dilution Buffer III (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled H<sub>2</sub>O.

### Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS, pH 7.2, 12.5mM β-glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

### [<sup>33</sup>P]-ATP Assay Cocktail

Prepare 250µM [<sup>33</sup>P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [<sup>33</sup>P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at -20°C.

### 10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at -20°C.

### Substrate (Catalog #: A16-58)

Axltide synthetic peptide substrate (KKSRGDYMTMQIG) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

## Assay Protocol

- Step 1.** Thaw [<sup>33</sup>P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active AXL, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 3.** In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
- Component 1.** 10µl of diluted Active AXL (Catalog #A34-11H)
  - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #A16-58)
  - Component 3.** 5µl distilled H<sub>2</sub>O (4°C)
- Step 4.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H<sub>2</sub>O.
- Step 5.** Initiate the reaction by the addition of 5 µl [<sup>33</sup>P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6.** After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7.** Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H<sub>2</sub>O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8.** Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9.** Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

### Calculation of [<sup>33</sup>P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [<sup>33</sup>P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

### Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of <sup>33</sup>P-ATP in cpm/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg)]\*[(Reaction Volume) / (Spot Volume)]

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# MATERIAL SAFETY DATA SHEET

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## Article 1 - Product Identification and Use

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**Product Name: AXL, Active**

**Catalog # A34-11H**

*This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.*

Manufacturer's Name:	SignalChem Pharmaceuticals Inc.
Street Address:	110-13120 Vanier Place
City, Prov. Postal Code:	Richmond, BC, V6V 2J2
Fax:	604-232-4601
EMERGENCY PHONE:	604-232-4600

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## Article 2 - Hazardous Ingredients

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NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

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## Article 3 - Physical Data

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This product consists of purified protein in sodium phosphate buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

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## Article 4 - Fire and Explosion Hazard

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NOT APPLICABLE

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## Article 5 - Reactivity Data

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NOT APPLICABLE

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## Article 6 - Toxicologically Data

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May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

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## Article 7 - Preventative Measures

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Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

\*\*\*\*\*MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES\*\*\*\*\*

- Wear protective equipment.
- Absorb on sand or vermiculite and place in closed containers for disposal.
- Observe all federal, state and local environmental regulations.

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## Article 8 - First Aid Measures

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- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
- If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
- In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.

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## Article 9 - Preparation

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Prepared By: Jun Yan

Phone #: 1-866-954-6273

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