

AXL (R499C), Active

Recombinant protein expressed in Sf9 cells

Catalog # A34-12BG

Lot # V2430-5

Product Description

Recombinant human AXL (R499C) (473-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The Axl gene accession number is [NM_021913](#).

Gene Aliases

UFO, JTK11

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF and 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.

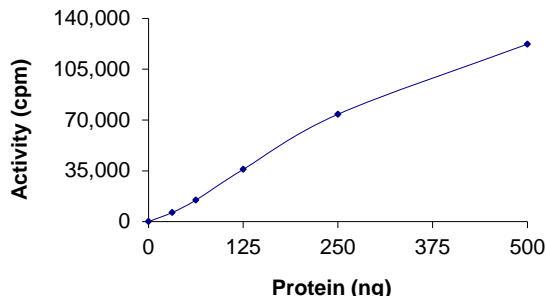
Scientific Background

AXL (R499C) is an arginine to cysteine mutation at 499 aa in AXL and appears as a somatic mutation in gastric adenocarcinoma samples. AXL is a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase which is a member of the TAM (TYRO3/AXL/MER) receptor tyrosine kinase subfamily (1). AXL represents a unique structure of the extracellular region that juxtaposes IgL and FNIII repeats and AXL transduces signals from the extracellular matrix into the cytoplasm by binding growth factors like vitamin K-dependent protein growth-arrest-specific gene 6 (2).

References

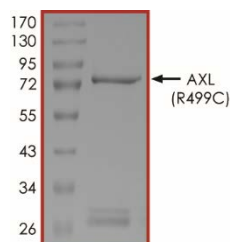
- O'Bryan, J. P.et.al: Axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. Molec. Cell. Biol. 11: 5016-5031, 1991.
- Varnum, B. C.et.al: Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6. Nature 373: 623-626, 1995.

Specific Activity



The specific activity of AXL (R499C) was determined to be **3.5 nmol /min/mg** as per activity assay protocol.

Purity



The purity of AXL (R499C) was determined to be **>80%** by densitometry, approx. MW **76 kDa**.

AXL (R499C), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	A34-12BG
Specific Activity	3.5 nmol/min/mg
Lot #	V2430-5
Purity	>80%
Concentration	0.1 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.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: A34-12BG)

Active AXL (R499C) (0.1µg/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 (R499C) 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 50ng/µl BSA solution.

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

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 25mM MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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 peptide substrate (KKSRRGDYMTMQIG) diluted in distilled H₂O to a final concentration of 1mg/ml.

Note: Poly (4:1 Glu, Tyr) peptide substrate (Catalog #P61-58) can also be used as a substrate for this target and shows better activity.

Assay Protocol

- Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active AXL (R499C), 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 (R499C) (Catalog # A34-12BG)
 - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog # A16-58)
 - Component 3. 5µl distilled H₂O (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 H₂O.
- Step 5. Initiate the reaction by the addition of 5 µl [³³P]-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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.