



cell signaling solutions

---

## Certificate of Analysis

10 Old Barn Road • Lake Placid, NY 12946  
Technical Support: T: 800 548-7853 • F: 518 523-4513  
email: techserv@upstate.com  
Sales Department: T: 800 233-3991 • F: 781 890-7738  
Licensing Dept.: 800 310-4659  
www.upstate.com

### Axl-tide

(Axl/Insulin Receptor substrate peptide)

Catalog # 12-516

Lot # 24560

**Product Description:** Synthetic peptide [KKSREGDYMTMQIG]. Use as a specific substrate for Axl (Catalog # 14-512) or Insulin Receptor (Catalog # 14-466). MW = 1514.96 Daltons.

**Purity:** >90% determined by HPLC.

**Application:** Use 250 $\mu$ M as the substrate for *in vitro* Axl or Insulin Receptor activity assay.

**Formulation:** 1mg peptide determined by amino acid analysis. Lyophilized powder.

**Rehydration:** Rehydrate in water or an aqueous buffer.

**Storage and Stability:** Stable for 2 years at 4°C from date of shipment. Upon reconstitution, aliquot to prevent multiple freeze-thaw cycles and store at -20°C.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

---

### Quality Control Testing and Research Applications

Protein Kinase Assay: This lot was tested by using 20ng purified Axl (Catalog # 14-512) to phosphorylate 250 $\mu$ M of the substrate peptide. Assay results show a 94-fold increase in phosphate transferred to the peptide over background.

---

### Kinase Assay Protocol

#### Stock Solutions:

1. **5X Reaction buffer:** 40mM MOPS, pH 7.0, 1mM EDTA.
2. **Enzyme Dilution Buffer (EDB):** 20mM MOPS, pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
3. **Axl, active** (Catalog #14-512): Dilute to 0.8-8ng/ $\mu$ l with EDB. Use 2.5 $\mu$ l per assay point.
4. **Axl-tide:** Prepare a 2.5mM stock. Use 2.5 $\mu$ l per assay point for a final assay concentration of 250 $\mu$ M per assay point.
5. **[ $\gamma$ -<sup>32</sup>P]ATP:** Stock 1mCi/100 $\mu$ l (3000Ci/mmol, obtained from PerkinElmer, Cat. # BLU002A). Make 10 $\mu$ l aliquots (100 $\mu$ Ci/vial). Before starting the assay, dilute an aliquot to 1 $\mu$ Ci/ $\mu$ l with 90 $\mu$ l of 75mM MgCl<sub>2</sub> and 500 $\mu$ M cold ATP in 20mM MOPS, pH 7.2, 25mM  $\beta$ -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol (Magnesium/ATP Cocktail, Catalog # 20-113).

#### Assay Procedure:

1. Add 5 $\mu$ l of 5X Reaction Buffer per assay.
2. Add 2.5 $\mu$ l of **Axl-tide** (250 $\mu$ M).
3. Add 2.5 $\mu$ l (2-20ng) of Axl, active.
4. Add 5 $\mu$ l of sterile, distilled water.
5. Add 10 $\mu$ l of the diluted [ $\gamma$ -<sup>32</sup>P]ATP solution.
6. Incubate for 10 minutes at 30°C.
7. Transfer a 20 $\mu$ l aliquot onto the center of a 2cm x 2cm P81 paper.
8. Wash the assay squares three times for 5 minutes with 0.75% phosphoric acid.
9. Wash the assay squares once for 5 minutes with acetone.
10. Transfer the assay squares to vial and add 1ml scintillation cocktail.
11. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain background control.

**Technical Note:** Allow the radiolabeled substrate to bind to the filter paper for 30 seconds before immersing the paper into a 50ml conical tube containing 40ml 0.75% phosphoric acid. Gently shake the assay squares for 5 minutes on a rotator. Discard the wash in a liquid radioisotope waste container, (dispose of per institutional regulations) and repeat the wash step twice. Wash the squares in 20ml of acetone for 5 minutes. Drain and add scintillation cocktail.