

# Axl Antibody

✓ 100 µl  
(10 Western mini-blot)



Cell Signaling  
TECHNOLOGY®

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new 08/04

Applications W, IP	Species Cross-Reactivity H, M, R, Hm, Mk	Molecular Wt. 138 kDa	Source Rabbit
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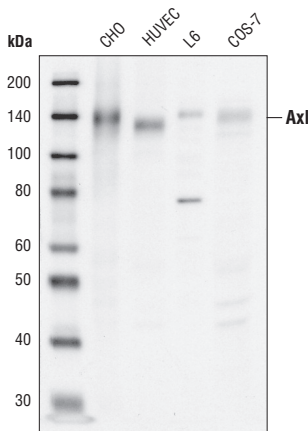
**Background:** Axl, Sky and Mer are members of a recently characterized family of receptor tyrosine kinases (RTKs) possessing an extracellular domain that resembles cell adhesion molecules and an intracellular conserved tyrosine kinase domain. These RTKs bind the vitamin K-dependent protein growth-arrest-specific gene 6 (Gas6), which is structurally related to the anticoagulation factor, protein S (1). Upon binding to its receptor, Gas6 activates phosphatidylinositol 3-kinase (PI3K) and its downstream targets S6K and Akt, as well as NF-κ B (2,3). A large body of evidence supports a role for Gas6/Axl signaling in cell growth and survival in normal and cancer cells (4).

**Specificity/Sensitivity:** Axl Antibody detects endogenous levels of Axl protein. The antibody does not cross-react with related proteins.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to sequences surrounding amino acid residue 740 of human Axl. Antibodies are purified by protein A and peptide affinity chromatography.

**Background References:**

- (1) Crosier, K.E. and Crosier, P.S. (1997) *Pathology* 29, 131–135.
- (2) Demarchi, F. et al. (2001) *J. Biol. Chem.* 276, 31738–31744.
- (3) Lee, W. P. et al. (2002) *Oncogene* 21, 329–336.
- (4) Bellosta, P. et al. (1997) *Oncogene* 15, 2387–2397.



Western blot analysis of extracts from various cell lines, using Axl Antibody.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

**Recommended Antibody Dilutions:**

Western blotting 1:1000  
Immunoprecipitation 1:50

**Companion Products:**

- Phototope®-HRP Western Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- LumiGLO® Reagent and Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry F—Flow cytometry E—ELISA D—DELFIAP®  
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Western Immunoblotting Protocol

**For Western blots, incubate membrane with diluted anti-body in 5% BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**A. Solutions and Reagents**

Note: Prepare solutions with Milli-Q or equivalently purified water.

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 1X SDS Sample Buffer:  
62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- A3. ■ Transfer Buffer:  
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- A4. ■ 10X Tris Buffered Saline (TBS):  
To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- A5. ■ Nonfat Dry Milk (weight to volume [w/v])
- A6. ■ Blocking Buffer:  
1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- A7. ■ Wash Buffer:  
1X TBS, 0.1% Tween-20 (TBS/T)
- A8. ■ Bovine Serum Albumin (BSA)
- A9. ■ Primary Antibody Dilution Buffer:  
1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- A10. ■ Phototope®-HRP Western Blot Detection System #7071:  
Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- A11. ■ Prestained Protein Marker, Broad Range (Premixed Format) #7720
- A12. ■ Biotinylated Protein Ladder Detection Pack #7727
- A13. ■ Blotting Membrane:  
This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

**B. Protein Blotting**

A general protocol for sample preparation is described below.

- B1. Treat cells by adding fresh media containing regulator for desired time.
- B2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- B3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- B4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- B5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- B6. Microcentrifuge for 5 minutes.
- B7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

*Note: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.*

- B8. Electrotransfer to nitrocellulose or PVDF membrane.

**C. Membrane Blocking and Antibody Incubations**

*Note: Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.*

- C1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- C2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- C3. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- C5. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- C7. Wash three times for 5 minutes each with 15 ml of TBS/T.

**D. Detection of Proteins**

- D1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

*Note: LumiGLO® substrate can be further diluted if signal response is too fast.*

- D2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

*Note: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.*

**A. Solutions and Reagents**

*Note: Prepare solutions with Milli-Q or equivalently purified water.*

A1. ■ 1X Phosphate Buffered Saline (PBS)

A2. ■ 1X Cell Lysis Buffer:

20 mM Tris (pH 7.5)

150 mM NaCl

1 mM EDTA

1 mM EGTA

1% Triton X-100

2.5 mM Sodium pyrophosphate

1 mM  $\beta$ -Glycerolphosphate

1 mM  $\text{Na}_3\text{VO}_4$

1  $\mu\text{g}/\text{ml}$  Leupeptin

*Note: CST recommends adding 1 mM PMSF before use\*.*

A3. ■ Transfer Buffer:

25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)

A4. ■ Protein A Agarose Beads:

(Can be stored for 2 weeks at 4°C.) Add 5 ml of 1X PBS to 1.5 g of protein A agarose beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS.

A5. ■ 3X SDS Sample Buffer:

187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

**B. Preparing Cell Lysates**

- B1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- B2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- B3. Remove PBS and add 0.5 ml 1X ice-cold cell lysis buffer plus 1 mM PMSF\* to each plate (10 cm) and incubate the plate on ice for 5 minutes.
- B4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
- B5. Sonicate on ice four times for 5 seconds each.
- B6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at -80°C.

**C. Immunoprecipitation**

- C1. Take 200  $\mu\text{l}$  cell lysate and add primary antibody; incubate with gentle rocking overnight at 4°C.
- C2. Add protein A agarose beads (20  $\mu\text{l}$  of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
- C3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500  $\mu\text{l}$  of 1X cell lysis buffer. Keep on ice during washes.
- C4. Resuspend the pellet with 20  $\mu\text{l}$  3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
- C5. Heat the sample to 95–100°C for 2–5 minutes.
- C6. Load the sample (15–30  $\mu\text{l}$ ) on SDS-PAGE gel (12–15%).
- C7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).