

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human and mouse Axl when phosphorylated at Y779.
Source	Polyclonal Rabbit IgG
Purification	Antigen and protein A Affinity-purified
Immunogen	Phosphopeptide containing human Axl Y779 site
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

APPLICATIONS

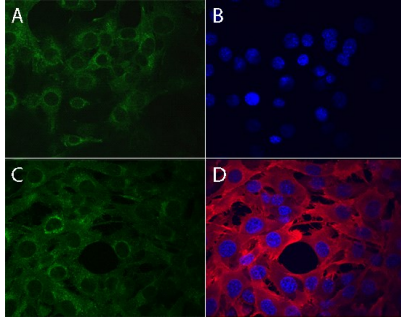
Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p>	<p>Detection of Human Phospho-Axl (Y779) by Western Blot. Western blot shows lysates of A172 human glioblastoma cell line untreated (-) or treated (+) with WI38 human lung cell line conditioned media (WI38 CM) for 15 minutes. PVDF membrane was probed with 1 µg/mL of Human Phospho-Axl (Y779) Antigen Affinity-purified Polyclonal Antibody, followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Phospho-Axl (Y779) at approximately 140 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunohistochemistry</p>	<p>Phospho-Axl (Y779) in Human Stomach Cancer Tissue. Axl phosphorylated at site Y779 was detected in immersion fixed paraffin-embedded sections of human stomach cancer tissue using Human Phospho-Axl (Y779) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2228) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue). Specific labeling was localized to the cytoplasm of epithelial cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.</p>
----------------------------	---	------------------------------------	--

Immunocytochemistry



Phospho-Axl (Y779) in A172 Human Cell Line. Axl phosphorylated at Y779 (panels B, D) and total Axl (panels A, C) were assessed in immersion fixed A172 human glioblastoma cells incubated with (panels C, D) or without (panels A, B) pervanadate. Phospho-Axl was detected using Rabbit Anti-Human Phospho-Axl (Y779) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2228) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red, panels B, D); Catalog # NL004) and counterstained using DAPI (blue). Total Axl was detected using Goat Anti-Human Axl Antigen Affinity-purified Polyclonal Antibody (Catalog # AF154). Cells were stained using the NorthernLights™ 493-conjugated Anti-Goat IgG Secondary Antibody (green, panels A, C); Catalog # NL003). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Axl (Ufo, Ark), Dtk (Sky, Tyro3, Rse, Brt), and Mer (human and mouse homologues of chicken c-Eyk) constitute a subfamily of the receptor tyrosine kinases (1, 2). The extracellular domains of these proteins contain two Ig-like motifs and two fibronectin type III motifs. This characteristic topology is also found in neural cell adhesion molecules and in receptor tyrosine phosphatases. The human Axl cDNA encodes an 887 amino acid (aa) precursor that includes an 18 aa signal sequence, a 426 aa extracellular domain, a 21 aa transmembrane segment, and a 422 aa cytoplasmic domain. The extracellular domains of human and mouse Axl share 81% aa sequence identity. A short alternately spliced form of human Axl is distinguished by a 9 aa deletion in the extracellular juxtamembrane region. These receptors bind the vitamin K-dependent protein growth arrest specific gene 6 (Gas6) which is structurally related to the anticoagulation factor protein S. Binding of Gas6 induces receptor autophosphorylation and downstream signaling pathways that can lead to cell proliferation, migration, or the prevention of apoptosis (3). This family of tyrosine kinase receptors is involved in hematopoiesis, embryonic development, tumorigenesis, and regulation of testicular functions.

References:

1. Yanagita, M. (2004) *Curr. Opin. Nephrol. Hypertens.* **13**:465.
2. Nagata, K. *et al.* (1996) *J. Biol. Chem.* **271**:30022.
3. Holland, S. *et al.* (2005) *Canc. Res.* **65**:9294.