Human Axl Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF154

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Axl in direct ELISAs and Western blots. In direct ELISAs, less than 25% cross-reactivity with recombinant mouse Axl is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Axl Glu33-Pro440 Accession # AAA61243
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 either lyophilized or as a 0.2 μm filtered solution in PBS.

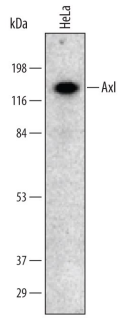
APPLICATIONS

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
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Knockout Validated	Axl is specifically detected in A431 human epithelial carcinoma parental cell line but is not detectable in Axl knockout A431 cell line.	
Blockade of Receptor-ligand Interaction	In a functional ELISA, 3-30 µg/mL of this antibody will block 50% of the binding of 20 ng/mL of biotinylated Recombinant Human Gas6, lacking the Gla domain, to immobilized Recombinant Human Axl Fc Chimera (Catalog # 154-AL) coated at 2 µg/mL (100 µL/well). At 100 µg/mL, this antibody will block >90% of the binding.	

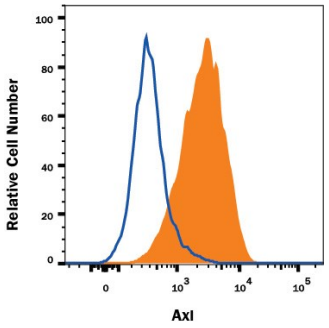
DATA

Western Blot



Detection of Human Axl by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line. PVDF membrane was probed with 1.0 µg/mL of Goat Anti-Human Axl Antigen Affinity-purified Polyclonal Antibody (Catalog # AF154) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for Axl as an approximately 140 kDa glycoprotein (as indicated). This experiment was conducted using Immunoblot Buffer Group 2.

Flow Cytometry



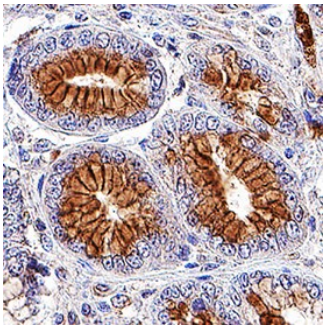
Detection of Axl in A431 Human Cell Line by Flow Cytometry. A431 human epithelial carcinoma cell line was stained with Goat Anti-Human Axl Affinity-Purified Polyclonal Antibody (Catalog # AF154, filled histogram) or normal goat IgG control antibody (Catalog # Catalog # AB-108C, open histogram) followed by APC-conjugated anti-Goat IgG Secondary Antibody (Catalog # Catalog # F0108). View our protocol for Staining Membrane-associated Proteins.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Human Axl Antibody

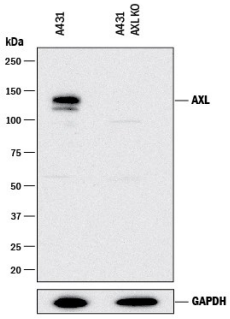
Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF154

Immunohistochemistry



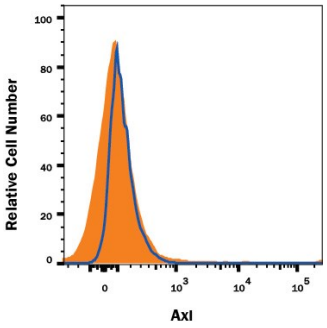
Axl in Human Stomach. Axl was detected in immersion fixed paraffin-embedded sections of human stomach using Goat Anti-Human Axl Antigen Affinity-purified Polyclonal Antibody (Catalog # AF154) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to plasma membranes of epithelial cells in gastric glands. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Knockout Validated



Western Blot Shows Human Axl Specificity by Using Knockout Cell Line. Western blot shows lysates of A431 human epithelial carcinoma parental cell line and Axl knockout A431 cell line (KO). PVDF membrane was probed with 1 µg/mL of Goat Anti-Human Axl Antigen Affinity-purified Polyclonal Antibody (Catalog # AF154) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for Axl at approximately 150 kDa (as indicated) in the parental A431 cell line, but is not detectable in knockout A431 cell line. GAPDH (Catalog # Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Knockout Validated



Axl Specificity is Shown by Flow Cytometry in Knockout Cell Line. Axl knockout A431 human epithelial carcinoma cell line was stained with Goat Anti-Human Axl Affinity-Purified Polyclonal Antibody (Catalog # AF154, filled histogram) or normal goat IgG control antibody (Catalog # Catalog # AB-108C, open histogram) followed by APC-conjugated anti-Goat IgG Secondary Antibody (Catalog # Catalog # F0108). No staining was observed in the Axl knockout A431 cell line. View our protocol for Staining Membrane-associated Proteins.

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.** • 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. **22**:30022.
3. Holland, S. *et al.* (2005) Canc. Res. **65**:9294.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449