Human Phospho-Axl (Y779) Antibody

Monoclonal Mouse IgG_1 Clone # 713610

Catalog Number: MAB6965

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Phospho-Axl (Y779) in direct ELISAs and Western blots.
Source	Monoclonal Mouse IgG_1 Clone # 713610
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Phosphopeptide containing the 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.

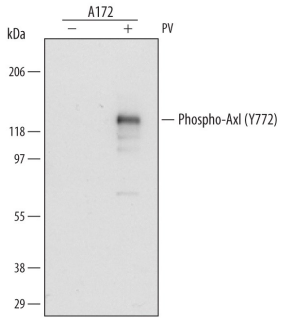
APPLICATIONS

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	0.2 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
Simple Western	4 µg/mL	See Below

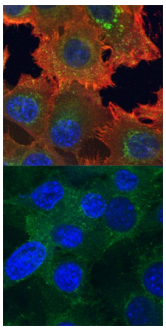
DATA

Western Blot



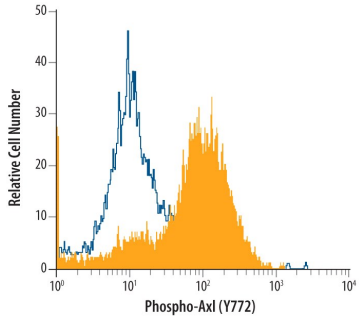
Detection of Human Phospho-Axl (Y779) by Western Blot. Western blot shows lysates of A172 human glioblastoma cell line untreated (-) or treated (+) with 1 mM Pervanadate (PV) for 30 minutes. PVDF membrane was probed with 0.2 µg/mL of Mouse Anti-Human Phospho-Axl (Y779) Monoclonal Antibody (Catalog # MAB6965) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). 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.

Immunocytochemistry



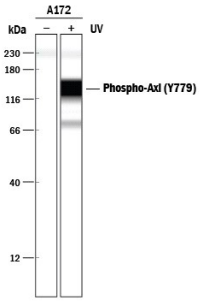
Phospho-Axl (Y779) in A172 Human Cell Line. Axl phosphorylated at Y779 and total Axl were assessed in immersion fixed A172 human glioblastoma cells incubated with (upper panel) or without (lower panel) the phosphatase inhibitor pervanadate at 100 µM for 5 minutes. Phospho-Axl was detected using Mouse Anti-Human Phospho-Axl (Y779) Monoclonal Antibody (Catalog # MAB6965) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) 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; Catalog # NL003). Specific staining was localized to cytoplasm and cell surfaces. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry



Detection of Phospho-Axl (Y779) in A172 Human Cell Line by Flow Cytometry. A172 human glioblastoma cell line was unstimulated (open histogram) or treated with 100 µM pervanadate for 5 minutes, then stained with Mouse Anti-Human Phospho-Axl (Y779) Monoclonal Antibody (Catalog # MAB6965, filled histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with Triton® X-100.

Simple Western



Detection of Human Phospho-Axl (Y779) by Simple Western™. Simple Western lane view shows lysates of A172 human glioblastoma cell line untreated (-) or treated (+) with 1 mM Pervanadate (PV) for 30 minutes, loaded at 0.2 mg/mL. A specific band was detected for Phospho-Axl (Y779) at approximately 150 kDa (as indicated) using 4 µg/mL of Mouse Anti-Human Phospho-Axl (Y779) Monoclonal Antibody (Catalog # MAB6965). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

*Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
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.

Human Phospho-Axl (Y779) Antibody

Monoclonal Mouse IgG_1 Clone # 713610

Catalog Number: MAB6965

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. Phosphorylation of Tyrosine 779 provides a docking site for p85 subunits of PI 3-Kinase (4).

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.
4. Weinger, J.G. *et al.* (2008) J. Neurochem. **106**:134.

PRODUCT SPECIFIC NOTICES

Triton is a registered trademark of Union Carbide Corp.