Human/Mouse/Rat PDK-1 Antibody

Monoclonal Mouse IgG_{2A} Clone # 650308

Catalog Number: MAB864

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat PDK-1 in Western blots.
Source	Monoclonal Mouse IgG_{2A} Clone # 650308
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	*E. coli*-derived recombinant human PDK-1 Asn411-Gln556 Accession # O15530
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.

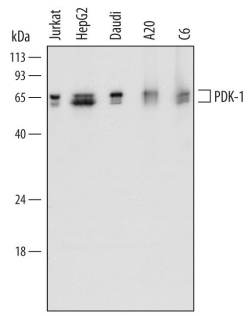
APPLICATIONS

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
Immunocytochemistry	5-25 µg/mL	See Below
Simple Western	20 µg/mL	Daudi human Burkitt's lymphoma cell line and HepG2 human hepatocellular carcinoma cell line

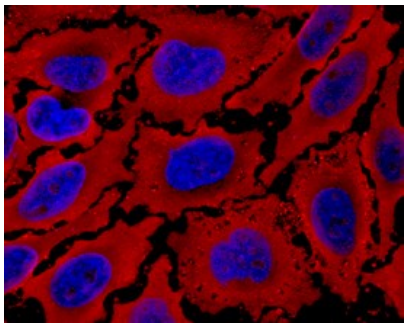
DATA

Western Blot



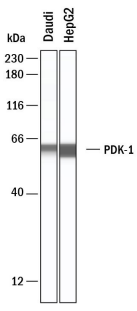
Detection of Human, Mouse, and Rat PDK-1 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line, HepG2 human hepatocellular carcinoma cell line, Daudi human Burkitt's lymphoma cell line, A20 mouse B cell lymphoma cell line, and C6 rat glioma cell line. PVDF Membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse/Rat PDK-1 Monoclonal Antibody (Catalog # MAB864) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). Specific bands were detected for PDK-1 at approximately 58-68 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



PDK-1 in HeLa Human Cell Line. PDK-1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Mouse Anti-Human/Mouse/Rat PDK-1 Monoclonal Antibody (Catalog # MAB864) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (yellow; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Simple Western



Detection of Human PDK-1 by Simple Western™. Simple Western shows lysates of Daudi human Burkitt's lymphoma cell line and HepG2 human hepatocellular carcinoma cell line, loaded at 0.5 mg/ml. A specific band was detected for PDK-1 at approximately 60 kDa (as indicated) using 20 µg/mL of Mouse Anti-Human/Mouse/Rat PDK-1 Monoclonal Antibody (Catalog # MAB864). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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Human/Mouse/Rat PDK-1 Antibody

Monoclonal Mouse IgG_{2A} Clone # 650308

Catalog Number: MAB864

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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

PDK-1 (3-phosphoinositide-dependent protein kinase, gene name PDPK1) is a 58 -68 kDa, 556 amino acid (aa) monomeric protein of the AGC serine/threonine kinase family. It is activated by phosphorylation in the presence of PtdIns(3,4,5) P3 or PtdIns(3,4) P2. Akt, S6 kinases, PKA and PKC-ζ are reported PDK-1 substrates. Through Akt, PDK-1 mediates many of the intracellular actions of insulin. Within the region used as an immunogen, human PDK-1 shares 98% aa identity with mouse and rat PDK-1. One reported isoform has an alternate start site at aa 50, while another lacking aa 238-263 is predicted to be catalytically inactive.

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