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Certificate of Analysis

Product: Affinity Purified Anti-PDK-1 (3-Phosphoinositide-Dependant Protein Kinase-1) (Rabbit)

Code: 600-401-411

Lot #: 11920

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.3 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C or below prior to opening. For extended storage, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening product.

Background Information: PDK-1 (3-Phosphoinositide-Dependant Protein Kinase-1) phosphorylates AGC kinases. PDK-1 activates conventional PKC and PKCz (zeta) through phosphorylation of critical threonine residues in the activation loop. PDK-1 also phosphorylates Protein Kinase B (PKB) at threonine 308 in the presence of phosphatidylinositol-3,4,5-trisphosphate. Active Akt inactivates Glycogen Synthase Kinase-3 (GSK3), eventually leading to the dephosphorylation and activation of glycogen synthase, and the stimulation of glycogen synthesis. Because of the role that PDK1 plays in insulin-induced glycogen synthesis and PKC activation, it is a potentially important target for metabolic drug research.

Application Note(s): This affinity purified antibody has been tested for use in ELISA and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band ~60 kDa in size corresponding to human PDK-1 protein by western blotting in the appropriate cell lysate or extract. Although untested, this reagent may be useful for immunohistochemistry and immunoprecipitation. Reactivity in other immunoassays is unknown.

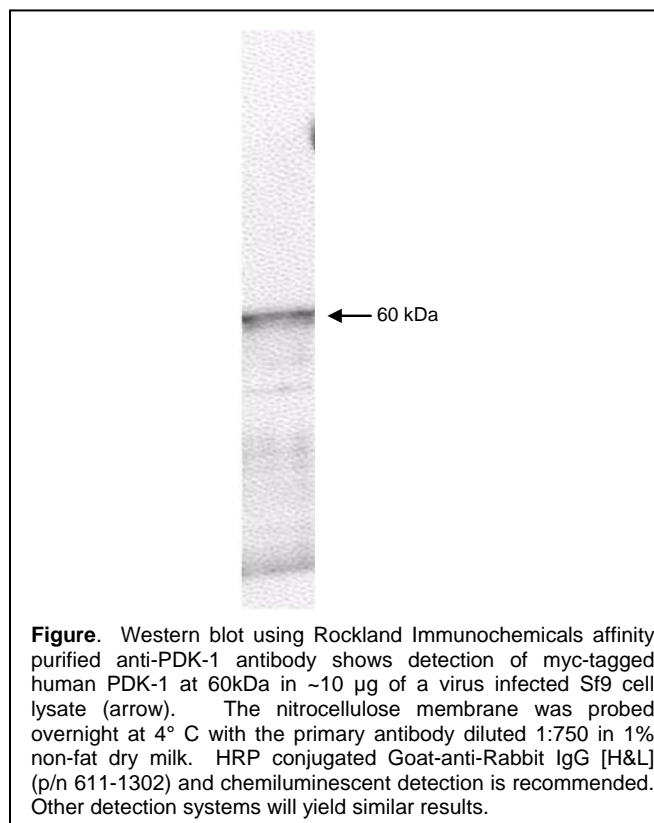


Figure. Western blot using Rockland Immunochemicals affinity purified anti-PDK-1 antibody shows detection of myc-tagged human PDK-1 at 60kDa in ~10 µg of a virus infected Sf9 cell lysate (arrow). The nitrocellulose membrane was probed overnight at 4° C with the primary antibody diluted 1:750 in 1% non-fat dry milk. HRP conjugated Goat-anti-Rabbit IgG [H&L] (p/n 611-1302) and chemiluminescent detection is recommended. Other detection systems will yield similar results.

Recommended Dilutions:

ELISA	1:100,000
WESTERN BLOT	1:500 - 1:2,000
IMMUNOHISTOCHEMISTRY	User Optimized
OTHER APPLICATIONS	User Optimized

Purity and Specificity: This affinity purified antibody is directed against human PDK-1. The product was affinity purified from monospecific antiserum by immunoaffinity chromatography. A BLAST analysis was used to suggest cross-reactivity with PDK-1 protein from mouse, rat and dog based on 92% homology with the immunizing sequence. Reactivity against homologues from other sources is unknown.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a region near the C-terminal of human PDK-1 protein.

Relevant Link: NCBI [NP_002604](#)

General References:

[Biondi RM, Komander D, Thomas CC, Lizcano JM, Deak M, Alessi DR, van Aalten DM.](#) (2002) High resolution crystal structure of the human PDK1 catalytic domain defines regulatory phosphopeptide docking site. *EMBO J.* **15**;21(16):4219-28.

[Fujita N, Sato S, Ishida A, Tsuruo T.](#) (2002) Involvement of Hsp90 in signaling and stability of 3 phosphoinositide dependent kinase 1. *J Biol Chem.* **22**;277(12):10346-53.

[Park J, Hill MM, Hess D, Brazil DP, Hofsteenge J, Hemmings BA.](#) (2001) Identification of tyrosine phosphorylation sites on 3 Phosphoinositide dependent protein kinase 1 and their role in regulating kinase activity. *J Biol Chem.* **5**;276(40):37459-71.

[Scheid MP, Marignani PA, Woodgett JR](#) (2002) Multiple phosphoinositide 3 kinase dependent steps in activation of protein kinase B. *Mol Cell Biol.* **22**(17):6247-60.

[Wick MJ, Wick KR, Chen H, He H, Dong LQ, Quon MJ, Liu F.](#) (2002) Substitution of the autophosphorylation site Thr516 with a negatively charged residue confers constitutive activity to mouse 3 Phosphoinositide dependent protein kinase 1 in cells. *J Biol Chem.* **10**;277(19):16632-8.

Related Products:

#611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#611-132-122	IRDye® 800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#611-145-122	DyLight™ 680 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#B501-0500	BLOTTO (500 g)
#BSA-30	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer) (500 ml)
#B304	NORMAL GOAT SERUM (NGS) (10 ml)
#KIA-003	MaxTag™ Anti-RABBIT IgG Kit for Immunoblotting
#MB-070	Blocking Buffer for Fluorescent Western Blotting

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.