ROCKLAND
Immunochemicals for Research
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Certificate of Analysis

Product: Affinity Purified Anti-Cyclin-Dependent Kinase 9 (CDK9) pT29 [Rabbit]

Code: 600-401-996 **Lot #** 19230

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

Background Information: CDK9 (PITALRE) (also known as cyclin-dependent kinase 9, Serine/threonine-protein kinase PITALRE, C-2K and Cell division cycle 2-like protein kinase 4) is a member of the cyclin-dependent protein kinase (CDK) family. CDK family members are highly similar to the gene products of *S. cerevisiae* cdc28 and *S. pombe* cdc2 and are known as important cell cycle regulators. CDK9 (PITALRE) interacts with a conserved domain in the TRAF-C region of the tumor necrosis factor signal transducer TRAF2. This kinase was also found to be a component of the multiprotein complex TAK/P-TEFb, which is an elongation factor for RNA polymerase II-directed transcription and functions by phosphorylating the C-terminal domain of the largest subunit of RNA polymerase II. This protein forms a complex with, and is regulated by, its regulatory subunit, cyclin T or cyclin K. HIV-1 Tat protein was found to interact with this protein and cyclin T, which suggests a possible involvement of this protein in AIDS. Tat stimulates human HIV-1 viral transcription elongation. This suggests that cyclin T1/CDK9(PITALRE) is one of the HIV-1 required host cellular cofactors generated during T cell activation. Cyclin T1/CDK9(PITALRE) has been shown to interact with Tat to restore Tat activation in HeLa nuclear extracts depleted of P-TEFb. CDK9(PITALRE) activity and cyclin T1 are essential for activation of viral transcription when tethered to the heterologous Rev response element RNA via the regulator of expression of virion Rev. CDK9 (PITALRE) is a ubiquitously expressed nuclear protein. CDK9 kinase activity is regulated by autophosphoryaltion of CDK9 at T29 and T186. T29 phosphorylation is uniquely induced by the HTLV-1 viral oncoprotein Tax.

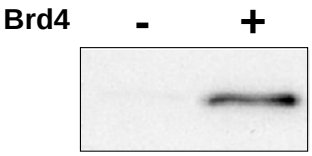

Figure. Western blot using Rockland's affinity purified anti-CDK9 pT29 antibody shows detection of phosphorylated CDK9. 100 ng of purified P-TEFb, which contains CDK9 and its regulatory cyclin T1 subunit, was incubated with ATP in the presence or absence of Brd4, a protein known to induce CDK9 phosphorylation at T29. The primary antibody was used at a 1:1000 dilution. *Personal Communication, J. Brady, NCI, Bethesda, MD.*

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 approximately 42 kDa in size corresponding to phosphorylated CDK9 protein by western blotting in the appropriate cell lysate or extract. This phospho-specific polyclonal antibody reacts with human CDK9 pT29 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide.

Recommended Dilutions:

ELISA	1:5,000 - 1:24,000
WESTERN BLOT	1:200 - 1:2,000
IMMUNOHISTOCHEMISTRY	User Optimized
OTHER APPLICATIONS	User Optimized

Purity and Specificity: This product was affinity purified from monospecific antiserum by immunoaffinity chromatography using phospho-peptide coupled to agarose beads followed by solid phase adsorption against nonphospho-peptide. This antibody is specific for human CDK9 protein phosphorylated at T29. A BLAST analysis was used to suggest cross-reactivity with CDK9 from human, mouse and rat based on 100% homology with the immunizing sequence. Cross-reactivity with CDK9 from other sources has not been determined.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to residues surrounding T29 in the human CDK9 protein.

Relevant Links: NCBI NP_001252 Swiss-Prot P50750

Related Products:

#100-401-167	Anti-cdk9 (PITALRE) (RABBIT)
#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
#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	***Max*Tag™** Anti-RABBIT IgG Kit for Immunoblotting
#MB-070	Blocking Buffer for Fluorescent Western Blotting

General References:

Zhou, M., Lu, H., Park, H., Wilson-Chiru, J., Linton, R. and Brady, J.N. (2006) Tax Interacts with P-TEFb in a Novel Manner To Stimulate Human T-Lymphotronic Virus Type 1 Transcription. *J. Virol.* **80** (10): 4781-4791.

Jang, M.K., Mochizuki, K., Zhou, M., Jeong, H.-S., Brady, J.N. and Ozato, K. (2005) Bromodomain Protein Brd4 is a Positive Regulatory Component of P-TEFb and Stimulates RNA Polymerase II Dependent Transcription. *Mol. Cell* **19**: 523-534.

Liu, H. and Herrmann, C.H. (2005) Differential localization and expression of the Cdk9 42k and 55k isoforms. *J. Cell. Physiol.* **203** (1): 251-260.

Peng, J., Zhu, Y., Milton, J.T. and Price, D.H. (1998). Identification of multiple cyclin subunits of human P-TEFb. *Genes Dev*. **12** (5): 755-762.

Zhou, Q., Chen, D., Pierstorff, E. and Luo, K. (1998) Transcription elongation factor P-TEFb mediates Tat activation of HIV-1 transcription at multiple stages. *EMBO J.* **17**(13): 3681-3691.

USDA Certification: 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.

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.