

Certificate of Analysis**Product:** Anti-CaM Kinase IV (Rabbit)**Code:** 100-401-883**Lot #:** 16340**Size:** 100 µg**Physical State:** Lyophilized**Protein Concentration:** 70 mg/ml by Refractometry**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 2° to 8° C prior to restoration. Reconstitute with 0.1 ml of deionized water (or equivalent). 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 restoration.

Background: CaM Kinase IV (also known as CAM kinase-GR, CAMK4 and CaMK IV) is a calcium/ calmodulin-dependent protein kinase belonging to a proposed calcium-triggered signaling cascade. This kinase may be involved in the transcriptional regulation of microtubule dynamics. In vitro, CaMK IV phosphorylates CREB1, CREBBP, PRM2, MEF2A, MEF2D and STMN1/OP18. CaMK IV may also be involved in spermatogenesis and may play a role in the consolidation/ retention of hippocampus-dependent long-term memory. CaMK IV must be phosphorylated to be maximally active and is phosphorylated by CAMKK1 or CAMKK2. In addition autophosphorylation of the N-terminus is required for full activation. Autophosphorylation of Ser-336 allows the kinase to switch to a Ca(2+)/calmodulin-independent state. Most likely the kinase is inactivated by the serine/ threonine protein phosphatase 2A. CaMK IV is a monomer that is located within the cytoplasm and nucleus and substantial localization occurs in certain neuronal nuclei. In spermatids CaMK IV is associated with chromatin and the nuclear matrix. CaMK IV is also specifically expressed in epithelial ovarian cancer tissue.

Application Note(s): This antiserum has been tested for use in Immunohistochemistry, ELISA and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band ~ 52 kDa in size corresponding to CaM Kinase IV by western blotting in the appropriate cell lysate or extract.

Recommended Dilutions:

ELISA	1:5,000 - 1:25,000
WESTERN BLOT	1:500 - 1:2,000
IMMUNOHISTOCHEMISTRY	1:500
OTHER APPLICATIONS	User Optimized

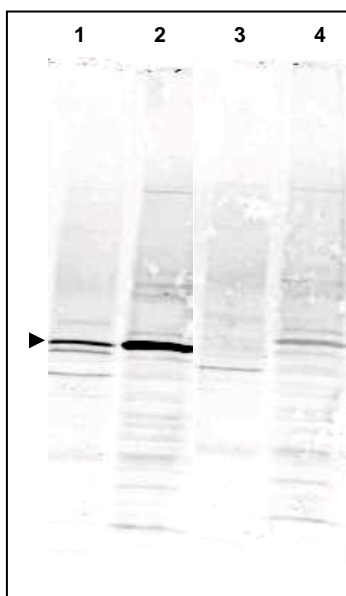
e-mail: info@rockland-inc.com

Figure. Western blot using Rockland's Anti-CaM Kinase IV antibody shows detection of a band ~52 kDa corresponding to CaM Kinase IV (arrowhead) in various preparations. Staining of rat brain lysate is shown in lane 1. Jurkat cell lysate staining is shown in lane 2. Specific reactivity is blocked in both lysates when antibody is preincubated with immunizing peptide (lanes 3 and 4 respectively). Approximately 35 µg of each lysate was separated by 4-20% SDS-PAGE and transferred onto nitrocellulose. CaM Kinase IV was similarly detected on lysates from mouse brain (not shown). After blocking the membrane was probed with the primary antibody diluted to 1:1,000 for 2h at room temperature followed by washes and reaction with a 1:10,000 dilution of IRDye™ 800 conjugated Gt-a-Rabbit IgG [H&L] MX (611-132-122)™ for 45 min at room temperature. IRDye™ 800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

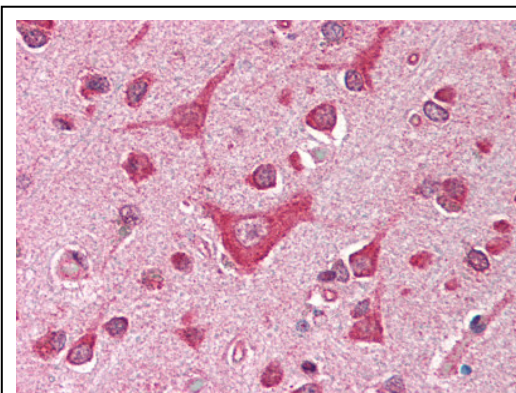


Figure 2. Immunohistochemistry. Rockland Immunochemicals' Anti-CAMK4 antibody was diluted 1:500 to detect CAMK4 in human brain cortex tissue. Tissue was formalin fixed and paraffin embedded. No pre-treatment of sample was required. The image shows the localization of antibody as the precipitated red signal, with a hematoxylin purple nuclear counter stain.

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Purity and Specificity: This antiserum is directed against human CaM Kinase IV protein. The product was delipidated, defibrinated followed by buffering and clarification. A BLAST analysis was used to suggest reactivity with this protein from human, mouse, rat, dog, bovine and chimpanzee based on 100% homology for the immunogen sequence. Cross reactivity with CaM Kinase IV protein from zebrafish, chicken and frog may occur as this sequence only varies by one or two amino acid residues (~89% homology). Cross reactivity with CaM Kinase IV homologues from other sources has not been determined.

Relevant Link(s): NCBI Link [Q16566](#) (Human) [P08414](#) (Mouse) [P13234](#) (Rat)

Immunogen: This antiserum was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 305-323 of Human CaM Kinase IV protein.

General References:

Kitani, T., Okuno, S. and Fujisawa, H. (1994) cDNA cloning and expression of human calmodulin-dependent protein kinase IV. *J. Biochem.* **115** (4), 637-640.

Bland, M.M., Monroe, R.S. and Ohmstede, C.A. (1994) The cDNA sequence and characterization of the Ca²⁺/calmodulin-dependent protein kinase-Gr from human brain and thymus. *Gene* **142** (2), 191-197.

Mosialos, G., Hanessian, S.H., Jawahar, S., Vara, L., Kieff, E. and Chatila, T.A. (1994) A Ca²⁺/calmodulin-dependent protein kinase, CaM kinase-Gr, expressed after transformation of primary human B lymphocytes by Epstein-Barr virus (EBV) is induced by the EBV oncogene LMP1. *J. Virol.* **68** (3), 1697-1705.

Jones, D.A., Glod, J., Wilson-Shaw, D., Hahn, W.E. and Sikela, J.M. (1991) cDNA sequence and differential expression of the mouse Ca²⁺/calmodulin-dependent protein kinase IV gene. *FEBS Lett.* **289** (1), 105-109.

Sikela, J.M., Law, M.L., Kao, F.T., Hartz, J.A., Wei, Q. and Hahn, W.E. (1989) Chromosomal localization of the human gene for brain Ca²⁺/calmodulin-dependent protein kinase type IV. *Genomics* **4** (1), 21-27.

Related Products:

#600-401-873	Affinity Purified Anti- CaM Kinase II (Rabbit)
#100-401-883	Affinity Purified Anti- CaM Kinase IV (Rabbit)
#W09-001-370	Jurkat Whole Cell Lysate in SDS-PAGE Sample Buffer
#W09-000-365	293 Whole Cell Lysate in SDS-PAGE Sample Buffer
#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 SOLUTION 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

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