abcam

Product datasheet

Anti-CaMKII alpha (phospho T286) antibody ab5683

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Overview

Product name	Anti-CaMKII alpha (phospho T286) antibody
Description	Rabbit polyclonal to CaMKII alpha (phospho T286)
Host species	Rabbit
Specificity	It does cross-react with CAMKII beta protein
Tested applications	**Suitable for:** IHC-FoFr, ICC/IF, WB
Species reactivity	**Reacts with:** Rat
Immunogen	Synthetic peptide corresponding to CaMKII alpha (phospho T286).

General notes

Calcium/calmodulin-dependent protein kinase II alpha (CaM Kinase II alpha) is a 50 kDa member of CaM Kinase II family of serine-threonine kinases that transduce Ca2+ signals to several target proteins, including ion channels and transcription activators. CaM Kinase II is predominantly expressed in two isoforms in the brain: alpha and beta. CaM Kinase II plays an important role in neuronal plasticity and memory formation, and exerts both calcium-calmodulin-dependent and -independent activities. Autophosphorylation of CaM Kinase II alpha on threonine 286 allows the kinase to switch from a calmodulin-dependent to a calmodulin-independent state, and is required for various cellular functions including hippocampal long-term potentiation (LTP), special learning, and hippocampus-dependent memory.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer	pH: 7.30

	Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol, 0.1% BSA
Purity	Immunogen affinity purified
Purification notes	The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated CaM Kinase II alpha. The final product is generated by affinity chromatography using a CaM Kinase II alpha-derived peptide that is phosphorylated at threonine 286.
Primary antibody notes	Calcium/calmodulin-dependent protein kinase II alpha (CaM Kinase II alpha) is a 50 kDa member of CaM Kinase II family of serine-threonine kinases that transduce Ca2+ signals to several target proteins, including ion channels and transcription activators. CaM Kinase II is predominantly expressed in two isoforms in the brain: alpha and beta. CaM Kinase II plays an important role in neuronal plasticity and memory formation, and exerts both calcium-calmodulin-dependent and -independent activities. Autophosphorylation of CaM Kinase II alpha on threonine 286 allows the kinase to switch from a calmodulin-dependent to a calmodulin-independent state, and is required for various cellular functions including hippocampal long-term potentiation (LTP), special learning, and hippocampus-dependent memory.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5683 in the following tested applications.

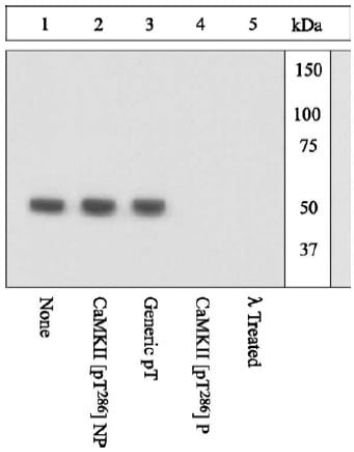
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-FoFr		1/300.
ICC/IF		Use a concentration of 5 µg/ml.
WB	★★★★★ **(4)**	1/1000. Detects a band of approximately 50 kDa.

Target

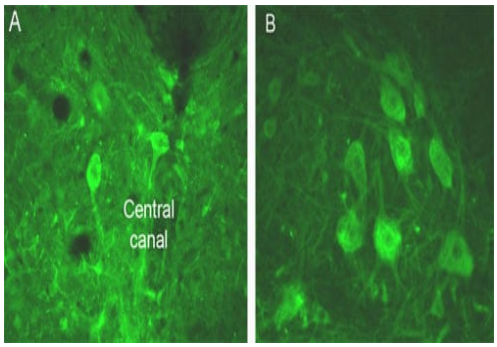
Function	CaM-kinase II (CAMK2) is a prominent kinase in the central nervous system that may function in long-term potentiation and neurotransmitter release. Member of the NMDAR signaling complex in excitatory synapses it may regulate NMDAR-dependent potentiation of the AMPAR and synaptic plasticity.
Sequence similarities	Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. CaMK subfamily. Contains 1 protein kinase domain.
Cellular localization	Cell junction > synapse > presynaptic cell membrane. Cell junction > synapse. Postsynaptic lipid rafts.

Images



Western blot - Anti-CaMKII alpha (phospho T286) antibody (ab5683)

Peptide Competition and Phosphatase Treatment: Rat brain lysates were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were either left untreated (1-4) or treated with lambda (ë) phosphatase (5) and blocked with a 5% BSA-TBST buffer for one hour at room temperature, then incubated with ab5683 antibody for two hours at room temperature in a 3% BSA-TBST buffer, following prior incubation with: no peptide (1, 5), the non-phosphopeptide corresponding to the immunogen (2), a generic phosphothreonine-containing peptide (3), or, the phosphopeptide immunogen (4). After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG HRP conjugate and bands were detected using the Pierce SuperSignal method. The data show that only the peptide corresponding to CaM Kinase II alpha [pT286] blocks the antibody signal. The data also show that phosphatase stripping eliminates the signal, verifying that the antibody is phospho-specific. Peptide Competiti

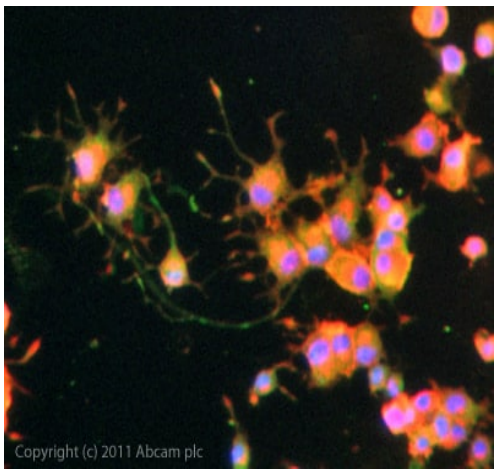


Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-CaMKII alpha (phospho T286) antibody (ab5683)

This image is courtesy of Sophie Pezet, Univ London Kings Coll, United Kingdom

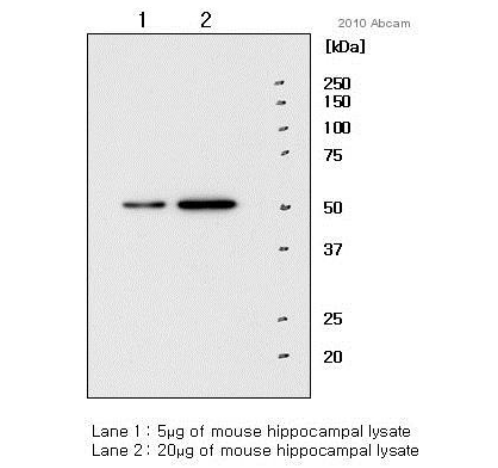
Immunofluorescent staining for CaMKII alpha phospho (T286) using ab5683 (1/300, incubated for 18 hours) in rat spinal cord. To induce CaMKII alpha phospho (T286) protein expression, a noxious stimulus was administered to the rat 5 minutes prior to 4% PFA perfusion fixation (this is a known paradigm for inducing phosphorylation CaMKII in some spinal neurons). The resulting immunofluorescent staining for CaMKII alpha phospho (T286) protein is observed in the cytoplasm of many dorsal horn spinal neurons (surrounding the central canal [A] or in the ventral horn [B]). Omition of primary antibody resulted in a lack of staining (data not shown).

Protocol details: Tissue was prepared by 4% paraformaldehyde cardiac perfusion fixation. Tissue was frozen on dry ice and then embedded in OCT compound and cut on cryostat. An antigen retrieval step was not neccesary for the IHC protocol.



Immunocytochemistry/ Immunofluorescence - Anti-CaMKII alpha (phospho T286) antibody (ab5683)

ICC/IF image of ab5683 stained PC12 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab5683, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-CaMKII alpha (phospho T286) antibody (ab5683)

This image is courtesy of an Abreview submitted by Dr. Byung-il Choi

Primary Antibody 1/1000 24 hours at 4°C
Secondary Antibody (1/5000): HRP-conjugated Goat polyclonal to rabbit IgG
Blocking step: 5% BSA for 12 hours at 4°C

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