√100 µl (10 western blots)



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rev. 09/30/13

## For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype	
W	H. M. R	60. 50 kDa	Rabbit laG**	
Endogenous	, ,	,		

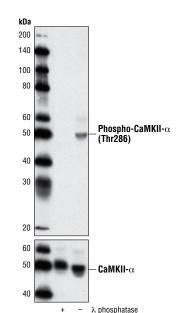
**Background:** CaMKII is an important member of the calcium/calmodulin-activated protein kinase family, functioning in neural synaptic stimulation and T cell receptor signaling (1,2). CaMKII has catalytic and regulatory domains. Ca<sup>2+</sup>/calmodulin binding to the CaMKII regulatory domain relieves autoinhibition and activates the kinase (3). The activated CaMKII further autophosphorylates at Thr286 to render the kinase constitutively active (3). The threonine phosphorylation state of CaMKII can be regulated through PP1/PKA. PP1 (protein phosphatase 1) dephosphorylates phospho-CaMKII at Thr286. PKA (protein kinase A) prevents phospho-CaMKII (Thr286) dephosphorylation through an inhibitory effect on PP1 (4).

**Specificity/Sensitivity:** Phospho-CaMKII (Thr286) (D21E4) Rabbit mAb recognizes endogenous levels of CamKII- $\alpha$  protein only when phosphorylated at Thr286. This antibody also recognizes endogenous levels of CamKII- $\beta$  and CamKII- $\gamma$  protein only when phosphorylated at Thr287.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr287 of human  $CamKII-\beta$  protein.

## **Background References:**

- (1) Hughes, K. et al. (2001) J Biol Chem 276, 36008-13.
- (2) Barria, A. et al. (1997) Science 276, 2042-2045.
- (3) Barkai, U. et al. (2000) Mol. Endocrinol. 14, 554-563.
- (4) Makhinson, M. et al. (1999) J Neurosci 19, 2500-10.



Western blot analysis of extracts from MKN-45 cells treated with λ-phosphatase or Forskolin #3828 (30 μM, 20 min) using Phospho-CaMKII (Thr286) (D21E4) Rabbit mAb (upper) and CaMKII-α (D10C11) Rabbit mAb #11945 (lower).

+ Forskolin

Entrez Gene ID #815, 816, 817, 818 UniProt ID #Q9UQM7, Q13554, Q13557, Q1355

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.* 

\*Species cross-reactivity is determined by western blot.

\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.

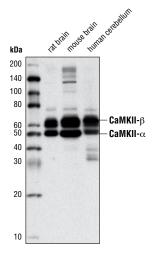
## **Recommended Antibody Dilutions:**

Western blotting

1:1000

For product specific protocols please see the web page for this product at www.cellsignal.com.

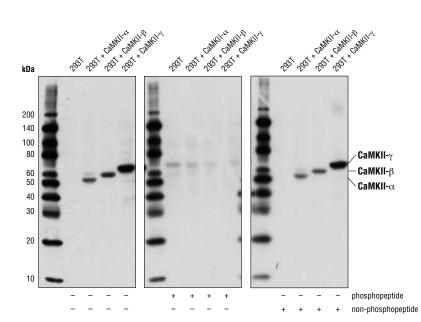
Please visit www.cellsignal.com for a complete listing of recommended companion products.



Western blot analysis of extracts from rat brain, mouse brain and human cerebellum using Phospho-CaMKII (Thr286) (D21E4) Rahhit mAh

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Tween® is a registered trademark of ICI Americas, Inc.



Western blot analysis of extracts from 293T cells, untransfected or transfected with specific CamKII proteins as indicated, using Phospho-CamKII (Thr286) (D21E4) Rabbit mAb. Antibody phosphospecificity was verified by preincubating the antibody in the absence of a peptide (-) or with either CamKII- $\alpha$  (Thr287) phosphopeptide (+) or CamKII- $\alpha$  (Thr287) non-phosphopeptide (+) prior to incubating the membrane.