Phospho-PLC Pathway Sampler Kit

E051020

Kits Includes	Cat.	Quantity	Application	Reactivity	Source
CaMKII (Phospho-Thr286) Antibody	E011287-1	50μg/50μl	WB	Human, Mouse, Rat	Rabbit
CREB (Phospho-Ser133) Antibody	E011052-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
MARCKS (Phospho-Ser158) Antibody	E011293-1	50μg/50μl	WB	Human, Mouse, Rat	Rabbit
PKCβ(Phospho-Thr641) Antibody	E011172-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
PLCγ1 (Phospho-Tyr783) Antibody	E011103-1	50μg/50μl	WB	Human, Mouse, Rat	Rabbit

CAMKII regulates numerous physiological functions, including neuronal synaptic plasticity through the phosphorylation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate (AMPA) receptors. Studies of the similar protein in rat suggest that this protein may function as a negative regulator of CaM-KII and may act to inhibit the phosphorylation of AMPA receptors. Potent and specific cellular inhibitor of CaM-kinase II (CAMK2). Traps Ca(2+)/calmodulin on CAMK2. May play an important role in the regulation of cell growth when overexpressed in colon adenocarcinoma LoVo cells. Traps Ca(2+)/calmodulin on CAMK2.

CREB1 gene encodes a transcription factor that is a member of the leucine zipper family of DNA binding proteins. This protein binds as a homodimer to the cAMP-responsive element, an octameric palindrome. The protein is phosphorylated by several protein kinases, and induces transcription of genes in response to hormonal stimulation of the cAMP pathway. Alternate splicing of this gene results in two transcript variants encoding different isoforms. This protein binds the cAMP response element (CRE), a sequence present in many viral and cellular promoters. **CREB** stimulates transcription on binding to the CRE. Transcription activation is enhanced by the TORC coactivators which act independently of Ser-133 phosphorylation. Implicated in synchronization of circadian rhythmicity.

MARCKS protein encoded by this gene is a substrate for protein kinase C. It is localized to the plasma membrane and is an actin filament crosslinking protein. Phosphorylation by protein kinase C or binding to calcium-calmodulin inhibits its association with actin and with the plasma membrane, leading to its presence in the cytoplasm. The protein is thought to be involved in cell motility, phagocytosis, membrane trafficking and mitogenesis. **MARCKS** is the most prominent cellular substrate for protein kinase C. This protein binds calmodulin, actin, and synapsin. **MARCKS** is a filamentous (F) actin cross-linking protein. Phosphorylation by PKC displaces MARCKS from the membrane. It also inhibits the F-actin cross-linking activity.

Protein kinase C (**PKC**) is a family of serine- and threonine-specific protein kinases that can be activated by calcium and second messenger diacylglycerol. **PKC** family members phosphorylate a wide variety of protein targets and are known to be involved in diverse cellular signaling pathways. **PKC** family members also serve as major receptors for phorbol esters, a class of tumor promoters. Each member of the **PKC** family has a specific expression profile and is believed to play a distinct role in cells. The protein encoded by this gene is one of the **PKC** family members. This protein kinase has been reported to be involved in many different cellular functions, such as B cell activation, apoptosis induction, endothelial cell proliferation, and intestinal sugar absorption. Studies in mice also suggest that this kinase may also regulate neuronal functions and correlate fear-induced conflict behavior after stress. Alternatively spliced transcript variants encoding distinct isoforms have been reported. This is a calcium-activated, phospholipid-dependent, serine- and threonine-specific enzyme. **PKC** is activated by diacylglycerol which in turn phosphorylates a range of cellular proteins. **PKC** also serves as the receptor for phorbol esters, a class of tumor promoters. May be considered as a novel component of the NF-kappa-B signaling axis responsible for the survival and activation of B-cells after BCR cross-linking (By similarity). Phosphorylation on Thr-500 of isoform beta-I, within the activation loop, renders it competent to autophosphorylate. Subsequent autophosphorylation of Thr-642 maintains catalytic competence, and autophosphorylation on Ser-661 appears to release the kinase into the cytosol. Similarly, isoform beta-II is autophosphorylated on 'Thr-640' and 'Ser-659', subsequent to phosphorylation on Thr-500. Autophosphorylated on other sites i.e. in the N-terminal and hinge regions have no effect on PKC activity (By similarity).

PLCG1 protein catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate. This reaction uses calcium as a cofactor and plays an important role in the intracellular transduction of receptor-mediated tyrosine kinase activators. For example, when activated by SRC, the encoded protein causes the Ras guanine nucleotide exchange factor RasGRP1 to translocate to the Golgi, where it activates Ras. Also, this protein has been shown to be a major substrate for heparin-binding growth factor 1 (acidic fibroblast growth factor)-activated tyrosine kinase. Two transcript variants encoding different isoforms have been found for this gene. **PLC**-gamma is a major substrate for heparin-binding growth factor 1 (acidic fibroblast growth factor)-activated tyrosine kinase.

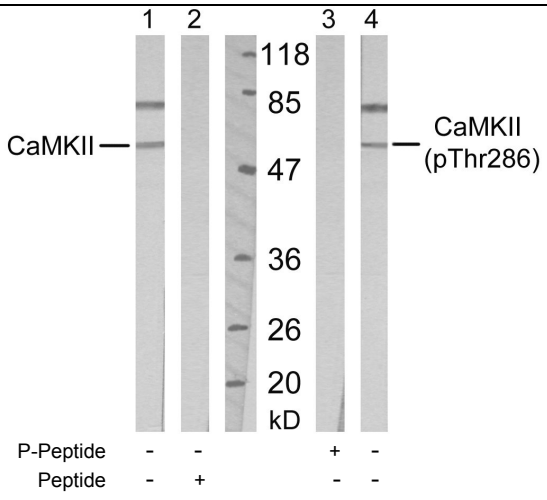
Phospholipases are a group of enzymes that hydrolyze phospholipids into fatty acids and other lipophilic molecules. PLC is subdivided into beta, gamma, delta, epsilon, zeta and eta subtypes, which catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG). IP3 and DAG both have important second messenger functions. PLC-beta is primarily activated by Gq/11 proteins and PLC-gamma is activated by phosphorylation in response to a variety of growth factor and immune system signals. Phospholipases are ubiquitously expressed and have diverse biological functions including roles in inflammation, cell growth, signaling and death and maintenance of membrane phospholipids. The receptor-mediated

activation of PLC-gamma-1 and PLC-gamma-2 involves their phosphorylation by tyrosine kinases in response to ligation of a variety of growth factor receptors and immune system receptors.

CaMKII (Phospho-Thr286) Antibody

E011287

Catalog Number: E011287-1, E011287-2
Amount: 50μg/50μl, 100μg/100μl
Swiss-Prot No. : Q9UQM7
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human CaMKII around the phosphorylation site of threonine 286 (Q-E-T^P-V-D).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: CaMKII (phospho-Thr286) antibody detects endogenous levels of CaMKII only when phosphorylated at threonine 286.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000
References: Pak JH, et al. Proc Natl Acad Sci U S A. 2000 Oct 10; 97(21): 11232-11237
Hudmon A, et al. J Cell Biol. Author manuscript; available in PMC 2006 May 7
Miller P, et al. PLoS Biol. 2005 Apr; 3(4): e107
Runyan JD, et al. Learn Mem. 2005 Mar; 12(2): 103-110.

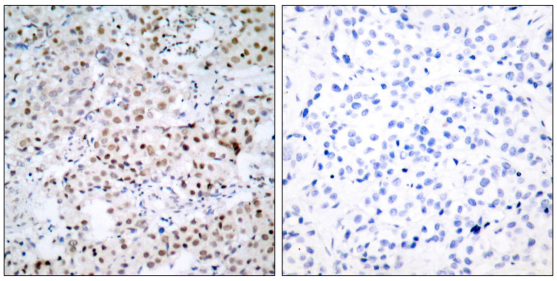


Western blot analysis of extract from Mouse brain tissue, using CaMKII (Ab-286) antibody (E021279, Lane 1 and 2) and CaMKII (phospho-Thr286) antibody (E011287, Lane 3 and 4).

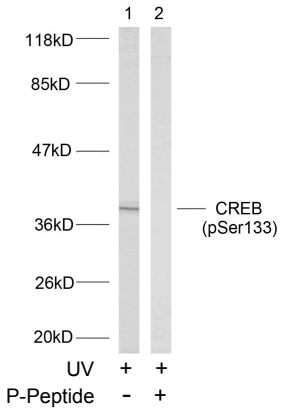
CREB (Phospho-Ser133) Antibody

E011052

Catalog Number: E011052-1, E011052-2
Amount: 50μg/50μl, 100μg/100μl
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human CREB around the phosphorylation site of serine 133 (R-P-S^{P}-Y-R).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatogramphy using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: CREB (phospho-Ser133) antibody detects endogenous levels of CREB only when phosphorylated at serine 133.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
Swiss-Prot No. : P16220
References:
Xing J, et al. (1998) Mol Cell Biol 18(4): 1946-55.
Tan Y, et al. (1996) **EMBO** J; 15(17): 4629-42.
Hao, M. et al. (1996) J. Biol. Chem. 271, 29380-29385.
Mayo LD, et al. (2001) Biol Chem; 276(27): 25184-9.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using CREB (phospho-Ser133) antibody (E011052).

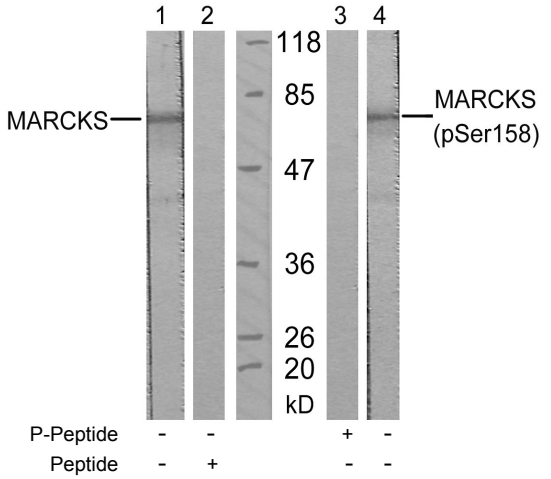


Western blot analysis of extracts from HeLa cells using CREB (phospho-Ser133) antibody (E011052).

E011293

MARCKS (Phospho-Ser158) Antibody

Catalog Number: E011293-1, E011293-2
Amount: 50μg/50μl, 100μg/100μl
Swiss-Prot No. : P29966
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human MARCKS around the phosphorylation site of serine 158 (R-F-S^{P}-F-K).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: MARCKS (phospho-Ser158) antibody detects endogenous levels of MARCKS only when phosphorylated at serine 158.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000
References: Pariser H, et al. Proc Natl Acad Sci U S A 2005 Aug 30; 102(35): 12407-12412
Nagumo H, et al. Biochem Biophys Res Commun 2001 Jan 26; 280(3): 605-609
Yamamoto H, et al. Arch Biochem Biophys 1998 Nov 15; 359(2): 151-159

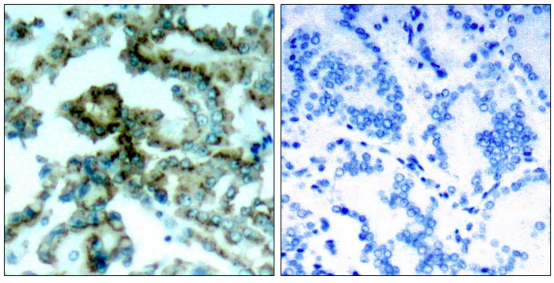


Western blot analysis of extract from starved NIH/3T3 cells, using MARCKS (Ab-158) antibody (E021285, Lane 1 and 2) and MARCKS (phospho-Ser158) antibody (E011293, Lane 3 and 4).

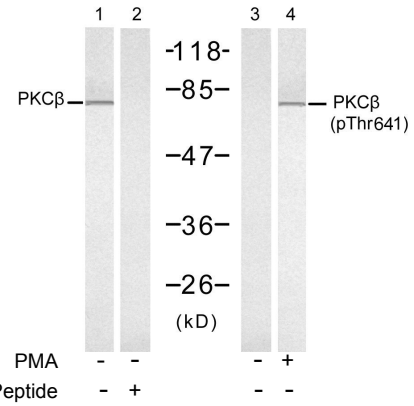
PKCβ (Phospho-Thr641) Antibody

E011172

Catalog Number: E011172-1, E011172-2
Amount: 50μg/50μl, 100μg/100μl
Swiss-Prot No. : P05771
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human PKCβ around the phosphorylation site of threonine 641 (E-L-T^P-P-T).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatogramphy using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: PKCβ (phospho-Thr641) antibody detects endogenous levels of PKCβ only when phosphorylated at threonine 641.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50-1:100
References: Zhang Y, et al. (2006) Mol Cell Biol ; 26: 6748-6761
Castoria G, et al. (2004) Mol Cell Biol ; 24: 7643-7653
Marcil J, et al. (1999) Biochem J ; 337:185-192
Bornancin F, et al. (1996) Curr Biol ; 6:1114-1123.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue, using PKCβ (phospho-Thr641) antibody (E011172).

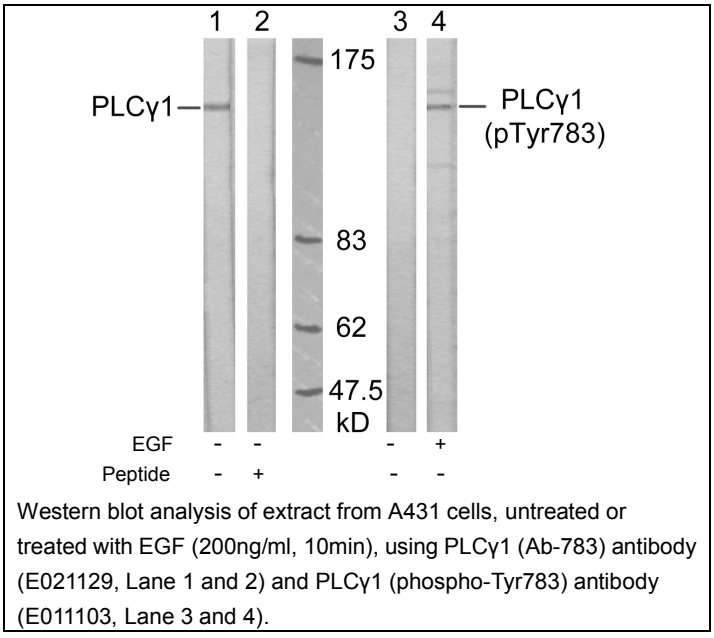


Western blot analysis of extracts from K562 cells, untreated or treated with PMA (1ng/ml, 10min), using PKCβ (Ab-641) antibody (E021184, Lane 1 and 2) and PKCβ (phospho-Thr641) antibody (E011172, Lane 3 and 4).

PLCγ1 (Phospho-Tyr783) Antibody

E011103

Catalog Number: E011103-1, E011103-2
Amount: 50μg/50μl, 100μg/100μl
Swiss-Prot No. : P19174
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human PLCγ1 around the phosphorylation site of tyrosine 783 (G-F-Y^P-V-E).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: PLCγ1 (phospho-Tyr783) antibody detects endogenous levels of PLCγ1 only when phosphorylated at tyrosine 783.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000
References: DeBell KE, et al. Mol Cell Biol. 1999 Nov; 19(11): 7388-7398.
Verí MC, et al. Mol Cell Biol. 2001 Oct; 21(20): 6939-6950.
Sette C, et al. EMBO J. 2002 Oct 15; 21(20): 5386-5395.



Western blot analysis of extract from A431 cells, untreated or treated with EGF (200ng/ml, 10min), using PLCγ1 (Ab-783) antibody (E021129, Lane 1 and 2) and PLCγ1 (phospho-Tyr783) antibody (E011103, Lane 3 and 4).