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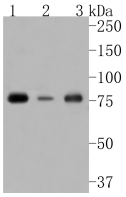
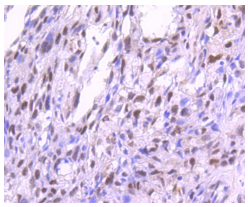
Phospho-PKC alpha (Thr638) Recombinant Rabbit mAb

Catalog Number:	bsm-52187R
Target Protein:	Phospho-PKC alpha (Thr638)
Concentration:	1mg/ml
Form:	Liquid
Host:	Rabbit
Clonality:	Recombinant
Clone No.:	4B3
Isotype:	IgG
Applications:	WB (1:500-2000), IHC-P (1:50-1:500), IHC-F (1:50-1:500), IF (1:50-1:500), Flow-Cyt (2ug/Test), ICC/IF (1:50)
Reactivity:	Human, Mouse, Rat (predicted:Pig, Cow, Chicken, Horse)
Predicted MW:	77 kDa
Entrez Gene:	5578
Swiss Prot:	P17252
Source:	KLH conjugated synthesised phosphopeptide derived from human PKC alpha around the phosphorylation site of Thr638: VL(p-T)PP.
Purification:	affinity purified by Protein A
Storage:	0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol. Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.
Background:	Protein Kinase c alpha (PKC alpha) is an 77 kDa member of the conventional group (cPKCs: sensitive to calcium, diacylglycerol, phosphatidylserine and phorbol esters) of the PKC family of serine/ threonine kinases that are involved in a wide range of physiological processes including mitogenesis, cell survival and transcriptional regulation. PKC alpha is an ubiquitously expressed PKC isozyme that has been implicated in the regulation of a broad range of cellular functions including proliferation, differentiation, development, migration, cell cell adhesion, cell extracellular matrix adhesion, and solute transport. The activation loop threonine (threonine 497 in PKC alpha) of conventional PKCs is phosphorylated by phosphoinositide dependent kinase 1 (PDK1). This phosphorylation is necessary for the autophosphorylation of threonine 638 in the carboxy terminus of PKC alpha, a step that is critical for regulating the rate of PKC alpha dephosphorylation and inactivation.

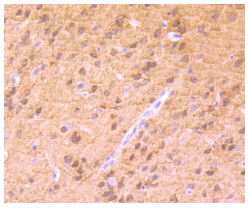
VALIDATION IMAGES



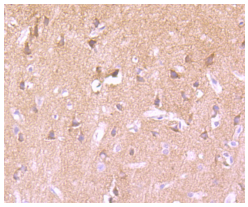
Sample: Lane 1: 293 cell lysate Lane 2: Hela cell lysate Lane 3: Jurkat cell lysate Primary: Anti-Phospho-PKC alpha (Thr638) (bsm-52187R) at 1/500 dilution Secondary: Goat Anti-Rabbit IgG - HRP at 1:5000 dilution Predicted band size: 77 kD Observed band size: 77 kD



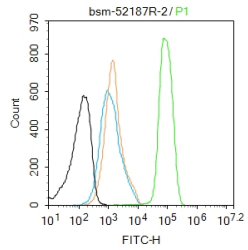
Paraformaldehyde-fixed, paraffin embedded (human breast carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-PKC alpha (Thr638)) Monoclonal Antibody, Unconjugated (bsm-52187R) at 1:50 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



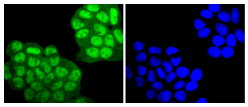
Paraformaldehyde-fixed, paraffin embedded (mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-PKC alpha (Thr638)) Monoclonal Antibody, Unconjugated (bsm-52187R) at 1:50 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-PKC alpha (Thr638)) Monoclonal Antibody, Unconjugated (bsm-52187R) at 1:50 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Blank control:MCF7. Primary Antibody (green line): Rabbit Anti-Phospho-PKC alpha (Thr638) antibody (bsm-52187R) Dilution: 2µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody : Goat anti-rabbit IgG-AF488 Dilution: 1µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 90% ice-cold methanol for 20 min at-20°C. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.



Hela cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (Phospho-PKC alpha (T638) in) monoclonal Antibody, Unconjugated (bsm-52187R) 1:50, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei.