Technical Data Sheet

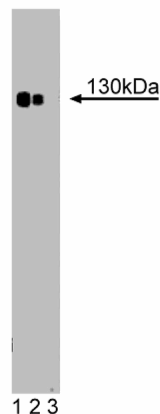
Purified Mouse Anti-Phospholipase Cβ4

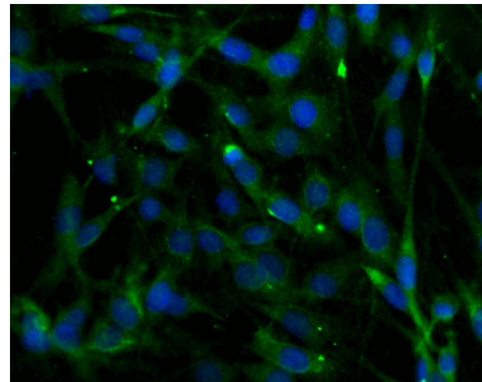
Product Information

Material Number:	**611540**
Alternate Name:	PLCβ4
Size:	50 µg
Concentration:	250 µg/ml
Clone:	56/Phospholipase Cβ4
Immunogen:	Human Phospholipase Cβ4 aa. 752-961
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Rat Tested in Development: Human, Mouse, Drosophila
Target MW:	130 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Phospholipase C (PLC) hydrolyzes inositol phospholipids into diacylglycerol and inositol 1,4,5-trisphosphate (IP3). Multiple distinct PLC isoenzymes have been identified and divided into three structural types: α, β, and γ. This classification is based primarily on the location of the conserved X and Y domains, whose structural integrity is essential for a functional catalytic core. The activation of PLCβ isoenzymes is uniquely regulated by G protein subunits, while PLCγ is activated following phosphorylation by protein tyrosine kinases. The β subfamily of PLC consists of at least four members: β1, β2, β3, and β4. PLCβ4 differs from the other members in that it is not activated by G protein βγ subunits, it is not found in the liver or kidney, and it is inhibited by ribonucleotides. Various isoforms of PLβC4 result from alternative splicing or proteolytic cleavage. PLCβ4 is expressed in retina and brain and knockout mice display ataxia and abnormalities in metabotropic glutamate receptor function in the cerebellum. Thus, PLCβ4 is primarily found in neuronal tissues where it is thought to be important in neurotransmitter signaling pathways.





Western blot analysis of Phospholipase Cβ4 on a rat pituitary lysate (left)*.* *Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the mouse anti-Phospholipase Cβ4 antibody.*

Immunofluorescent staining of C6 cells (Rat glioma; ATCC CCL-107) (right). *Cells were seeded in a 384-well collagen coated microplate (Material # 353962) at ~ 6,000 cells per well. After overnight incubation, cells were stained using the Triton-X 100 fix/perm protocol (see Recommended Assay Procedure) and the mouse anti-Phospholipase Cβ4 antibody. The second step reagent was Alexa Fluor® 488 goat anti-mouse Ig (Invitrogen). The image was taken on a BD Pathway™ 855 or 435 Bioimager using a 20x objective. This antibody also stained SH-SY5Y (Human neuroblastoma; ATCC CRL-2266) and SK-N-SH cells (Human neuroblastoma; ATCC HTB-11) using both the Triton-X 100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20°C.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Routinely Tested
Immunofluorescence	Tested During Development

Recommended Assay Procedure:

Western blot: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Bioimaging:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353962	BD Falcon™ 384-well Imaging Plate	1 box	test clone

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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Lee CW, Park DJ, Lee KH, Kim CG, Rhee SG. Purification, molecular cloning, and sequencing of phospholipase C-beta 4. *J Biol Chem.* 1993; 268(28):21318-21327.(Biology)