Technical Data Sheet

Purified Mouse Anti-Human PI3-Kinase C2β

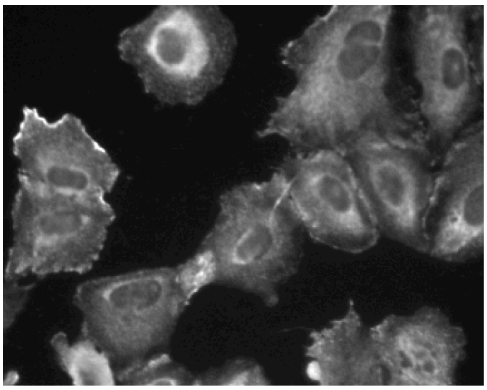
Product Information

Material Number:	**611342**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	22/PI3-K
Immunogen:	Human PI3-Kinase C2β aa. 16-209
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Target MW:	165 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Phosphatidylinositol (PtdIns) (3) kinase phosphorylates the D-3 position of the inositolring of PtdIns, producing PtdIns(3)P, PtdIns(3,4)P2, and PtdIns(3,4,5)P3. PI3-kinase is a heterodimer of an 85 kDa regulatory subunit (p85) and a 110 kDa catalytic subunit (p110). However, it is only one member of a larger family of proteins with similarity to the p110 subunit. These different PI3-kinase isoforms have been divided into three classes. Class I consists of p110α and p110ß which bind the p85 subunit and associate with receptor tyrosine kinases. Class II includes 68D and cpk from *Drosophila*, p170 and cpk-m from mouse, and C2α, C2ß (HsC2), and C2γ from human. These proteins phosphorylate PtdIns and PtdIns(4)P, but not PtdIns(4,5)P2, and each contain a C-terminal C2 domain that may negatively regulate the catalytic domain. Class III members only phosphorylate PtdIns to PtdIns(3)P and include the *S. cerevisiae* Vps34p and its human homologs. In humans, the class II PI3-kinases C2α and C2ß have similar catalytic, PI kinase, and C2 domains. However they differ in their N-terminal regions. In addition, C2ß has no cation specificity, while C2α prefers Mg2+-ATP for optimal phosphorylation.

Western blot analysis of PI3-Kinase C2β on a HeLa lysate. *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the PI3-Kinase C2β antibody.*



Immunofluorescent staining of A549 (ATCC CCL-185) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-PI3-Kinase C2β antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway 855 Bioimager using a 20x objective. This antibody also stained U-2 OS (ATCC HTB-96) and HeLa (ATCC CCL-2) cells using both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611449	HeLa Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
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.
6. Triton is a trademark of the Dow Chemical Company.

References

Arcaro A, Volinia S, Zvelebil MJ, et al. Human phosphoinositide 3-kinase C2beta, the role of calcium and the C2 domain in enzyme activity. *J Biol Chem.* 1998; 273(49):33082-33090. (Biology)