Technical Data Sheet

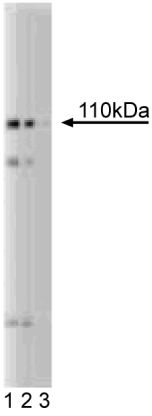
Purified Mouse Anti-PI4-Kinase β

Product Information

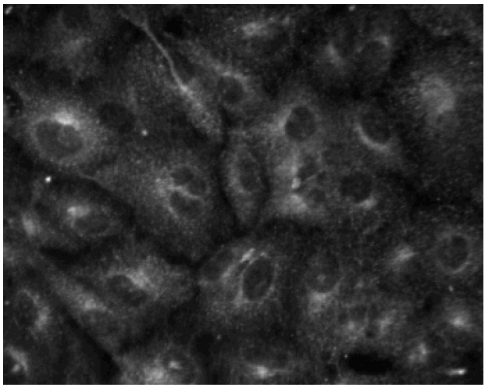
Material Number:	**611817**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	7/PI4-Kinase β
Immunogen:	Human PI4Kβ aa. 411-626
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human Tested in Development: Dog, Mouse, Rat
Target MW:	110 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Phosphoinositide turnover is a well established mechanism of intracellular signal transduction. Sequential phosphorylation of phosphatidylinositol (PtdIns) results inPtdIns-4-phosphate (PIP) and PtdIns-4,5-bisphosphate (PIP2). Phospholipase C (PLC)hydrolyzes PIP2 to inositol-1,4,5-trisphosphate (IP3) which stimulates release of intracellular Ca2+. PIP is generated by phosphorylation of PtdIns at the D4 position of the inositol ring. This event is mediated by the **P**td**I**ns **4-k**inases (PI4-K). These enzymes are divided into two types (II and III) based on their size and sensitivity to certain compounds. Although the PI4-Ks are abundantly distributed throughout the cell, activity is found primarily in association with membranous structures. Members of this family contain a lipid kinase unique domain and a C-terminal catalytic domain. Two mammalian PI4-Ks, PI4-Kα and PI4-Kß, have been identified. PI4-Kß is homologous to the yeast PI4-K, PIK1. Based on its size and sensitivity to wortmanin (a PI3-K inhibitor), PI4-Kß is classified as a type III enzyme. Although it is found in the cytosol and in association with the Golgi, the specific function of PI4-Kß is yet to be determined.



Western blot analysis of PI4-Kinase β on a HeLa lysate. *Lane 1: 1:10000, lane 2: 1:20000, lane 3: 1:40000 dilution of the PI4-Kinase β 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-PI4 Kinase β 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 HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells using both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

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

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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

Balla T, Downing GJ, Jaffe H, Kim S, Zolyomi A, Catt KJ. Isolation and molecular cloning of wortmannin-sensitive bovine type III phosphatidylinositol 4-kinases. *J Biol Chem.* 1997; 272(29):18358-18366. (Biology)

Meyers R, Cantley LC. Cloning and characterization of a wortmannin-sensitive human phosphatidylinositol 4-kinase. *J Biol Chem.* 1997; 272(7):4384-4390. (Biology)

Wong K, Meyers ddR, Cantley LC. Subcellular locations of phosphatidylinositol 4-kinase isoforms. *J Biol Chem.* 1997; 272(20):13236-13241. (Biology)