Technical Data Sheet

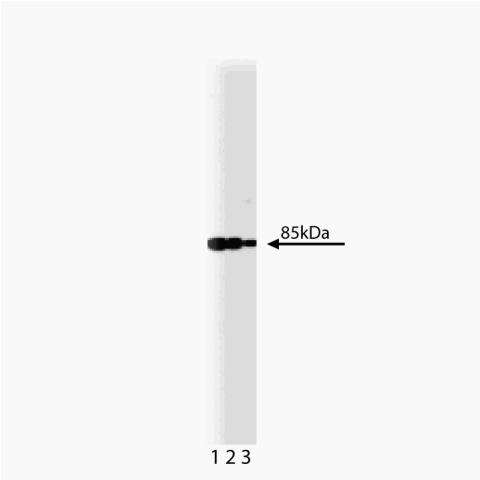
Purified Mouse Anti-PI3-Kinase

Product Information

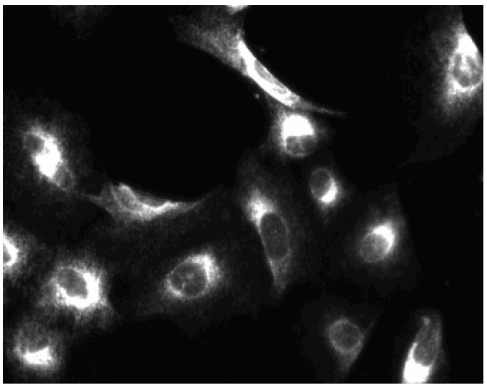
Material Number:	**610045**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	4/PI3-Kinase
Immunogen:	Human PI3-Kinase α subunit aa. 562-724
Isotype:	Mouse IgG2a
Reactivity:	QC Testing: Human Tested in Development: Dog, Rat, Mouse, Chicken
Target MW:	85 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

PI3-kinase phosphorylates the D-3 position of the inositol ring of phosphatidylinositol (PtdIns), PtdIns(4)P and PtdIns(4,5)P2 to produce the respective PI3-phosphorylated derivatives. PI3-kinase exists as a heterodimer of 85 kDa (p85) and 110 kDa (p110) subunits. The p85 subunit contains two SH2 domains and an SH3 domain. It associates with and serves as a substrate for activated growth factor receptor tyrosine kinases. p85 may serve as regulator of the catalytic subunit, p110, by acting as the link between PI3-kinase and the ligand-activated receptor. Two distinct forms of the p85 subunit have been described: 1) p85α, which binds tightly to the catalytic subunit, and 2) p85ß, a protein whose function is presently unknown. Both isoforms bind to activated receptors and serve as tyrosine kinase substrates.



Western blot analysis of PI3-Kinase on a A431 lysate. *Lane 1: 1:2500, lane 2: 1:5000, lane 3: 1:10000 dilution of the PI3-Kinase 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 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).*

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
Immunohistochemistry	Tested During Development
Immunoprecipitation	Tested During Development
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)
611447	A431 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

Cantley LC, Auger KR, Carpenter C, et al. Oncogenes and signal transduction. *Cell.* 1991; 64(2):281-302. (Biology)

Efendiev R, Yudowski GA, Zwiller J, et al. Relevance of dopamine signals anchoring dynamin-2 to the plasma membrane during Na+,K+-ATPase endocytosis. *J Biol Chem.* 2002; 277(46):44108-44114. (Clone-specific: Immunofluorescence, Immunoprecipitation, Western blot)

Kihara T, Shimohama S, Sawada H, et al. alpha 7 nicotinic receptor transduces signals to phosphatidylinositol 3-kinase to block A beta-amyloid-induced neurotoxicity. *J Biol Chem.* 2001; 276(17):13541-13546. (Clone-specific: Immunoprecipitation)

Nguyen MH, Ho JM, Beattie BK, Barber DL. TEL-JAK2 mediates constitutive activation of the phosphatidylinositol 3'-kinase/protein kinase B signaling pathway. *J Biol Chem.* 2001; 276(35):32704-32713. (Clone-specific: Immunoprecipitation, Western blot)

Zhang XA, Bontrager AL, Hemler ME.. Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific beta(1) integrins. *J Biol Chem.* 2001; 276(27):25005-25013. (Clone-specific: Immunofluorescence, Immunoprecipitation)