**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

Anti-PI3-kinase (p85α) mAb

Code No.	Clone	Subclass	Quantity	Concentration
MD-06-3	AB6	Mouse IgG1	100 µL	1 mg/mL

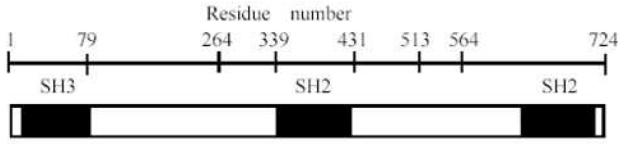
BACKGROUND: Phosphoinositide 3-kinase (PI3K) family proteins generate 3-phosphorylated phosphoinositides that act as second messengers downstream of numerous cellular receptors. This family control several cellular responses including cell growth, survival cytoskeletal remodeling and the trafficking of intracellular organelles in many different cell types. There are four subgroups (class IA, IB, II, III) of PI3K with distinct structure and substrate specificity. Class IA PI3K, which function downstream of activated tyrosine kinases, are heterodimers composed of a p110 catalytic subunit (α, β, or δ) and a tightly associated regulatory subunit (p85α, p55α, p50α, p85β, or p55γ). Each regulatory subunit can interchangeably associate with different catalytic subunits and shows a unique tissue distribution. p85α is the major regulatory subunit of class IA PI3Ks in most types of cells including immune cells.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0-Ag14 with Balb/c mouse splenocyte immunized with recombinant human p85α.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with p85α, which is regulatory subunit of PI3-kinase on Western blot, Immunoprecipitation and Immunocytochemistry. This antibody recognizes mutant p85α protein expressed in *E. coli* to be possessed in amino acid position '1-79' described below. This antibody does not react with p85β.



INTENDED USE:
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APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system

Immunoprecipitation; 1-5 µg/600 µL of cell extract from $5x10^6$ cells

Immunocytochemistry; 10 µg/mL
*Acetone, 80% methanol, 4% paraformaldehyde is available for fixation.

Immunohistochemistry; Not tested

Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

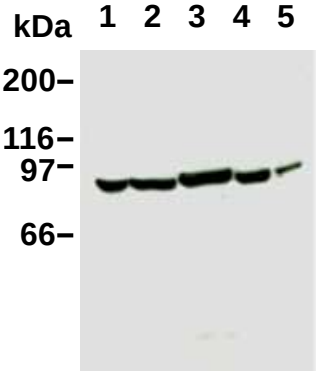
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, Raji, U937	WR19L	PC12, Rat-1
Reactivity on WB	+	+	+

REFERENCES:

1) Takeuchi, K. and Ito, F., *J. Biol. Chem.* **279**, 892-900 (2004) [WB]
2) Takeuchi, K., *et al.*, *J. Biol. Chem.* **276**, 26077-26083 (2001) [WB]
3) Takaishi, H., *et al.*, *PNAS* **96**, 11836-11841 (1999)
4) Kobayashi, M., *et al.*, *PNAS* **96**, 4874-4879 (1999)
5) Wakizaka, K., *et al.*, *Eur. J. Immunol.* **28**, 636-645 (1998) [WB]
6) Yamauchi, T., *et al.*, *Mol. Cell Biol.* **16**, 3074-3084 (1996) [IP]
7) Tobe, K., *et al.*, *J. Biol. Chem.* **270**, 5698-5701 (1995) [IP]
8) Tanaka, S., *et al.*, *Jpn. J. Cancer Res.* **84**, 279-289 (1993)

Clone AB6 is used in these references.



Western blot analysis of PI3-kinase (p85α) expression in Jurkat (1), Raji (2), U937 (3), WR19L (4) and PC12 (5) using MD-06-3.

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PROTOCOLS:

SDS-PAGE & Western Blotting

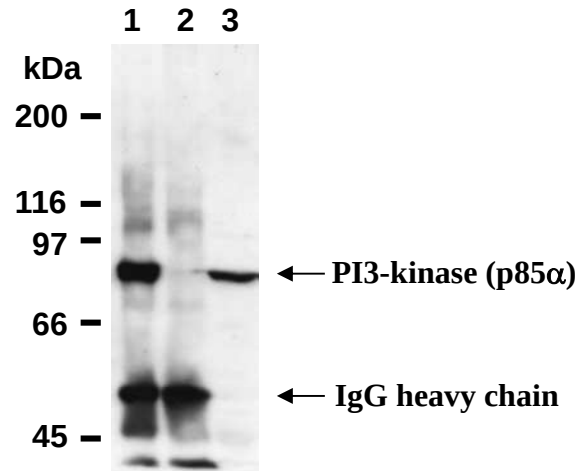
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 μL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji, U937, WR19L and PC12)

Immunoprecipitation

1) Collect the cultured cells from 75-cm^2 flask (containing about 5-10 x 10^6 cells).
2) Wash the cells 2 times with PBS and suspend with 1,200 μL of cold Lysis buffer [50 mM HEPES (pH 7.5), 250 mM NaCl, 0.1% NP-40, 5 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
3) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another fresh tube.
4) Add 50 μL of 50% protein G agarose beads in the supernatant. Incubate it at 4°C with rotating for 60 minutes.
5) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally divided into another two tubes.
6) Add Mouse IgG1 (isotype control) (MBL; code no. M075-3) or Anti-PI3-kinase (p85α) mAb (MBL; code no. MD-06-3) at the amount of as suggested in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gently agitation for 30-120 minutes at 4°C.
7) Add 20 μL of 50% protein G agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
8) Wash the beads 3-5 times with ice-cold IP buffer [10mM Tris-HCl (pH7.4), 150mM NaCl, 0.1% NP-40], centrifuge the tube at 2,500 x g for 10 seconds.
9) Resuspend the beads in 30 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 μL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Jurkat)



Immunoprecipitation of PI3-kinase (p85α) from Jurkat cells with MD-06-3 (1) or mouse IgG1 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with MD-06-3. Jurkat crude lysate was resolved in lane 3.*

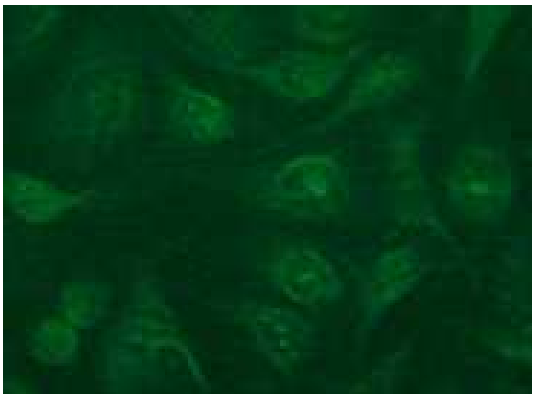
Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10^4 of cells per one well, then incubate in a CO_2 incubator for one night.)
2) Fix the cells by immersing the slide in acetone for 10 minutes on ice.
3) Wash the slide with PBS (5minutes x 3 times).
4) Cover the cells with blocking buffer (normal goat serum containing 1 mg/mL normal human IgG) for 10 minutes.
5) Tip off the blocking buffer, add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or

incubation condition is recommended if necessary.)

6) Wash the slide with PBS (5minutes x 3 times).
7) Add FITC conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
8) Wash the glass slide 5 times with PBS.
9) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
10) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HEp-II)



Immunocytochemical detection of human PI3-kinase (p85α) on HEp-II with MD-06-3.

RELATED PRODUCTS:

MH-11-3	Anti-Phosphotyrosine mAb (6D12)
MH-11-4	Anti-Phosphotyrosine mAb-FITC (6D12)
M075-3	Mouse IgG1 (isotype control) (2E12)