Monoclonal Anti-human/mouse Phospho-Akt (S473)-Alexa Fluor® 488

Catalog Number: IC7794G
Lot Number: ADIW01
100 Tests

Reagents Provided

Alexa Fluor® 488-conjugated mouse monoclonal anti-human/mouse Phospho-Akt (S473): Supplied as 10 μg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 545007

Isotype: mouse IgG_1

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

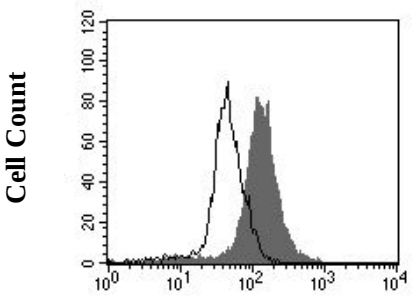
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing Phospho-Akt (S473) within a population and qualitatively determine the density of intracellular Phospho-Akt (S473) by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with a phospho-peptide containing the human Akt S473 site. The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Intracellular expression of Phospho-Akt (S473) is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515-545 nm.



Phospho-Akt(S473)-Alexa Fluor® 488

Jurkat cells, resting (open histogram) or treated with 100 nM Calyculin for 30 minutes (filled histogram), were stained with Alexa Fluor® 488-conjugated anti-human Phospho-Akt (S473) (Catalog # IC7794G).

Background Information

Akt, also known as protein kinase B (PKB), is a central kinase in such diverse cellular processes as glucose uptake, cell cycle progression, and apoptosis. Three highly homologous members define the Akt family: Akt1 (PKBα), Akt2 (PKBβ), and Akt3 (PKBγ). Akt1 is the most widely expressed family member and is frequently activated in a number of carcinomas, including breast, prostate, lung, pancreatic, liver, ovarian, and colorectal cancer. Akt1 is activated in a multistep process that involves the sequential phosphorylation of Thr450 by JNK kinases, Thr308 by PDK-1, and Ser473 by PDK-2 or mTORC2. Activated Akt1 phosphorylates a wide variety of cytosolic, nuclear, and mitochondrial substrates. Human Akt1 shares 98% aa sequence identity with mouse and rat Akt1. This antibody detects human and mouse Akt1, Akt2, and Akt3 when phosphorylated at S473, S474, and S472, respectively, by Western blot and direct ELISA (see the product datasheet for Catalog # MAB887).

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see Reagents Not Provided).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Up to 1 x 10^6 cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 5 μL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark.**
6. The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor® 488-labeled mouse IgG_1 antibody. This procedure may need to be modified, depending on the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

Legal

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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