Phospho-AKT1 (Ser473) Monoclonal Antibody (SDRNR), PE-Cyanine7, eBioscience™

Product Details	
Size	100 Tests
Species Reactivity	Human, Mouse
Host/Isotope	Mouse / IgG2a, kappa
Recommended Isotype Control	Mouse IgG2a kappa Isotype Control (eBM2a), PE-Cyanine7, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	SDRNR
Conjugate	PE-Cyanine7
Form	Liquid
Concentration	5 µL/Test
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2, with 0.2% BSA
Contains	0.09% sodium azide
Storage Conditions	4° C, store in dark, DO NOT FREEZE!
RRID	AB_2688172

Applications	Tested	Dilution	Published
Flow Cytometry (Flow)	✓	5 µL (0.06 µg)/test	

Product Specific Information

Description: This SDRNR monoclonal antibody recognizes human and mouse AKT (also known as Protein Kinase B (PKB)) when phosphorylated on S473. AKT is a serine/threonine protein kinase that plays a key role in multiple cellular processes including metabolism, proliferation, apoptosis/survival, and migration. There are three homologous isoforms of AKT: AKT1, AKT2, and AKT3. AKT is activated by binding of its pleckstrin homology (PH) domain to membrane phospholipids and by phosphorylation. Phosphorylation of AKT at T308 by PDK1 and at S473 is required for full activation of this kinase. AKT promotes cell survival by inhibiting apoptosis via phosphorylation and inactivation of several targets including Bad, Foxo1, c-Raf, and caspase-9. Deregulation of AKT has been implicated as a major contributing factor in many types of cancer. AKT is negatively regulated by the phosphatase PTEN as well as by the chemical inhibitor LY294002. Specificity of this SDRNR clone was determined by ELISA, flow cytometry, and western blotting.

Applications Reported: This SDRNR antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested: This SDRNR antibody has been pre-titrated and tested by intracellular staining followed by flow cytometric analysis of normal human peripheral blood cells. This can be used at 5 µL (0.06 µg) per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

Staining Protocol: All protocols work well for this monoclonal antibody. Use of Protocol A: Two-step protocol: intracellular (cytoplasmic) proteins allows for the greatest flexibility for detection of surface and intracellular (cytoplasmic) proteins. Use of Protocol B: One-step protocol: intracellular (nuclear) proteins is recommended for staining of transcription factors in conjunction with surface and phosphorylated intracellular (cytoplasmic) proteins. Protocol C: Two-step protocol: Fixation/Methanol allows for the greatest discrimination of phospho-specific signaling between unstimulated and stimulated samples, but with limitations on the ability to stain specific surface proteins (refer to "Clone Performance Following Fixation/Permeabilization" located in the Best Protocols Section under the Resources tab online). All Protocols can be found in the Flow Cytometry Protocols: "Staining Intracellular Antigens for Flow Cytometry Protocol" located in the Best Protocols Section under the Resources tab online.

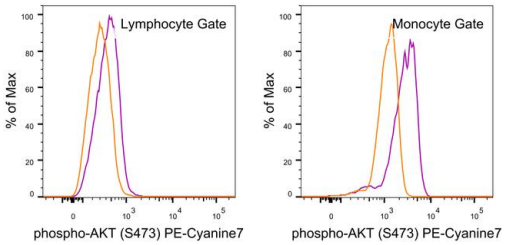
Light sensitivity: This tandem dye is sensitive to photo-induced oxidation. Please protect this vial and stained samples from light.

Fixation: Samples can be stored in IC Fixation Buffer (Product # 00-8222) (100 µL of cell sample + 100 µL of IC Fixation Buffer) or 1-step Fix/Lyse Solution (Product # 00-5333) for up to 3 days in the dark at 4°C with minimal impact on brightness and FRET efficiency/compensation. Some generalizations regarding fluorophore performance after fixation can be made, but clone specific performance should be determined empirically.

Excitation: 488-561 nm; Emission: 775 nm; Laser: Blue Laser, Green Laser, Yellow-Green Laser.

Filtration: 0.2 µm post-manufacturing filtered.

Product Images For Phospho-AKT1 (Ser473) Monoclonal Antibody (SDRNR), PE-Cyanine7, eBioscience™



Phospho-AKT1 (Ser473) Antibody (25-9715-42) in Flow
Normal human peripheral blood cells were unstimulated (orange histogram) or stimulated with Lipopolysaccharide (LPS) Solution (500X) (Product # 00-4976-03) (purple histogram), then intracellularly stained with Anti-Human/Mouse phospho-AKT (S473) PE-Cyanine7 using the Intracellular Fixation and Permeabilization Buffer Set (Product # 88-8824-00) and protocol. Cell in the lymphocyte (left) and monocyte (right) gates were used for analysis.