Phospho-S6 (Ser235, Ser236) Monoclonal Antibody (cupk43k), PerCP-eFluor™ 710, eBioscience™

Product Details	
Size	100 Tests
Species Reactivity	Human, Mouse
Published Species	Mouse
Host/Isotype	Mouse / IgG1, kappa
Recommended Isotype Control	Mouse IgG1 kappa Isotype Control (P3.6.2.8.1), PerCP-eFluor™ 710, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	cupk43k
Conjugate	PerCP-eFluor™ 710
Excitation/Emission Max	482/708 nm
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_2573858

Applications	Tested Dilution	Publications
Flow Cytometry (Flow)	5 µL (0.125 µg)/test	1 Publication

Product Specific Information

Description: This cupk43k monoclonal antibody recognizes human and mouse ribosomal protein S6 (also known as 40S ribosomal protein S6, phosphoprotein NP33, RPS6, RS6, S6) when phosphorylated on serine 235 (S235, human) and serine 236 (S236, mouse). Ribosomal protein S6 is a component of the 40S subunit of the ribosome and is phosphorylated at multiple sites following stimulation of cells by growth factors, tumor promoting agents, or mitogens. Phosphorylation of ribosomal protein S6 by p70S6K and PKDCD results in upregulation of the translation of RNA coding for proteins involved in cell cycle entry. Ribosomal protein S6 is dephosphorylated upon growth arrest.

The specificity of the cupk43k monoclonal antibody was determined by western blotting.

Applications Reported: This cupk43k antibody has been reported for use in intracellular staining followed by flow cytometric analysis.

Applications Tested: This cupk43k antibody has been pre-titrated and tested by intracellular staining and flow cytometric analysis of stimulated normal human peripheral blood cells. This can be used at 5 µL (0.125 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.

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 "Staining Intracellular Antigens for Flow Cytometry Protocol" located in the Best Protocols Section under the Resources tab online.

PerCP-eFluor® 710 emits at 710 nm and is excited with the blue laser (488 nm); it can be used in place of PerCP-Cyanine5.5. We recommend using a 710/50 bandpass filter, however, the 695/40 bandpass filter is an acceptable alternative. Please make sure that your instrument is capable of detecting this fluorochrome.

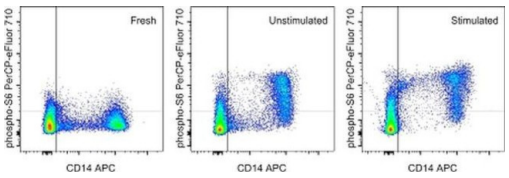
Light sensitivity: This tandem dye is sensitive to photo-induced oxidation. Please protect this vial and stained samples from light.

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 nm; Emission: 710 nm; Laser: Blue Laser.

Filtration: 0.2 µm post-manufacturing filtered.

Product Images For Phospho-S6 (Ser235, Ser236) Monoclonal Antibody (cupk43k), PerCP-eFluor™ 710, eBioscience™



Phospho-S6 (Ser235, Ser236) Antibody (46-9007-42) in Flow
Intracellular staining of freshly-harvested (left), unstimulated (middle), or 30-minute LPS-stimulated (right) normal human peripheral blood cells with Anti-Human CD14 APC (Product # 17-0149-42) and Anti-Human/Mouse phospho-S6 Ribosomal (S235/S236) PerCP-eFluor® 710, using the Intracellular Fixation and Permeabilization Buffer Set (Product # 88-8824-00) and protocol. Total cells were used for analysis.

1 Reference

Flow Cytometry (1)

Blood advances

Novel GM-CSF signals via IFN-R/IRF-1 and AKT/mTOR license monocytes for suppressor function.

"46-9007 was used in Flow cytometry/Cell sorting to study a time-dependent licensing process driven by GM-CSF in murine Ly6Chigh and human CD14+ monocytes that disables their inflammatory functions and promotes their conversion into suppressor cells."

Authors: Ribechini E,Hutchinson JA,Hergovits S,Heuer M,Lucas J,Schleicher U,Jordán Garrote AL,Potter SJ,Riquelme P,Brackmann H,Müller N,Raifer H,Berberich I,Huber M,Beilhack A,Lohoff M,Bogdan C,Eyrich M,Hermanns HM,Geissler EK,Lutz MB

Year
2017

Species
Mouse