Technical Data Sheet

Alexa Fluor® 647 Mouse anti-S6 (pS235/pS236)

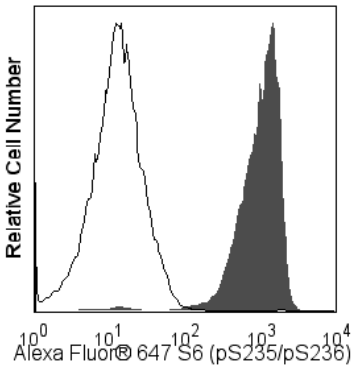
Product Information

Material Number:	**560435**
Alternate Name:	40S ribosomal protein S6; Phosphoprotein NP33; RPS6; RS6
Size:	50 tests
Vol. per Test:	20 µl
Clone:	N7-548
Immunogen:	Phosphorylated Human ribosomal protein S6 Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Predicted Reactivity: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

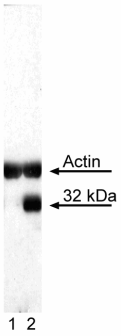
Description

Ribosomal protein S6 (~29 kDa calculated and ~32 kDa observed molecular weights) is a component of the 40S ribosomal subunit and belongs to the S6E family of ribosomal proteins. The S6 ribosomal protein plays a role in regulating the translation of RNAs and thus controlling the growth and proliferation of cells. S6 ribosomal protein phosphorylation, especially at multiple C-terminal serine residues S235, S236, S240, and S244, activates S6. The activated S6 ribosomal protein in turn upregulates the ribosomal translation of RNA species coding for other ribosomal proteins, peptide elongation factors and other proteins involved in cell cycle entry and progression. These phosphorylations are mediated by various kinases (e.g., p70S6K and PKCD) activated through cellular responses to growth factors, cytokines, tumor promoting agents, and mitogens. The S6 ribosomal protein can be dephosphorylated in growth-arrested cells.

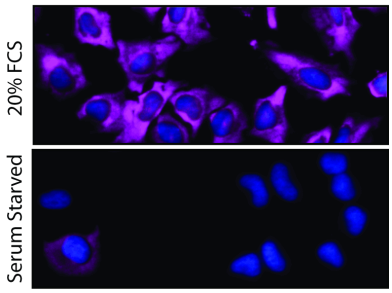
The N7-548 monoclonal antibody specifically detects the S6 ribosomal protein phosphorylated at S235 and S236.



Analysis of S6 (pS235/pS236) in activated human peripheral blood mononuclear cells (PBMC). *PBMC were isolated by density gradient centrifugation (Ficoll-Paque™ PLUS, Cat. No. 17-1440-02) and either left untreated (open histogram) or treated with PMA (Sigma-Aldrich, Cat. No. P8139) at 50 nM/10^6 cells for 30 minutes (shaded histogram). Cells were then fixed in BD Cytofix™ buffer (Cat. No. 554655) at 37°C for 10 minutes, then permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-S6 (pS235/pS236). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*



Western blot analysis of S6 (pS235/pS236). *The specificity of mAb N7-548 was confirmed by western blot analysis using unconjugated Mouse anti-S6 (pS235/pS236) antibody on lysates from untreated (lane 1) or PMA-treated (lane 2) PBMC. S6 (pS235/pS236) is identified as a band of 32 kDa, with increased intensity in the PMA-treated cells. Purified Mouse anti-Actin monoclonal antibody (Cat. No. 612656 or 612657) was the gel-loading control.*



Immunofluorescent staining of human cell line. *HeLa cells (ATCC CCL-2) were seeded in a 96-well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight serum starvation, some wells were cultured for 30 minutes with 20% fetal bovine serum (FBS, top panel) and others were not (lower panel). The cells were fixed with BD Cytofix™ buffer (Cat. No. 554655), permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050), stained with Alexa Fluor® 647 Mouse anti-S6 (pS235/pS236) (psedocolored magenta), and the nuclei were counterstained with Hoechst 33342 (pseudo colored blue). The images were captured on a BD Pathway™ 435 High-Content Bioimager System with a 20x objective and merged using BD Attovision™ software. The staining worked with the cold methanol and the Triton X-100 Perm/Wash protocols.*

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	PBMC	PMA	Cytofix	Perm I, II, or III	Upregulated expression
WB	Human	HEK 293	Serum starvation			no band observed
	Human	HEK 293	20% FBS			32-kDa band induced
	Human	HEK 293	20% FBS + S235/S236 phospho peptide			32-kDa band decreased
	Human	HEK 293	20% FBS + S235/S236 non-phospho peptide			32-kDa band not affected
	Human	HEK 293	20% FBS + S240 or S244 phospho peptide			32-kDa band not affected
	Mouse	NIH/3T3	PDGF			32-kDa band increased
	Human	PBMC	Untreated			32-kDa band
	Human	PBMC	PMA			32-kDa band increased

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Bioimaging	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
557885	Perm/Wash Buffer I	125 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. 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.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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