Technical Data Sheet

Alexa Fluor® 647 Mouse anti-S6 (pS244)

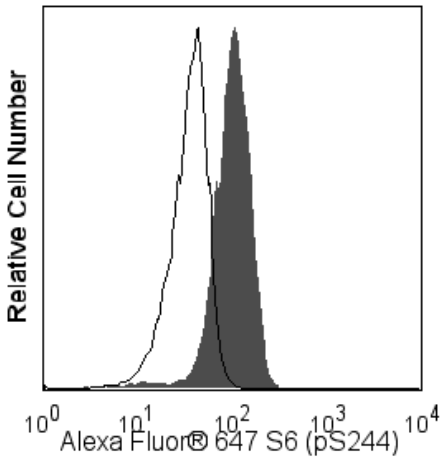
Product Information

Material Number:	**560465**
Alternate Name:	40S ribosomal protein S6; Phosphoprotein NP33; RPS6; RS6
Size:	50 tests
Vol. per Test:	20 µl
Clone:	N5-676
Immunogen:	Phosphorylated Human ribosomal protein S6 Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Predicted due to immunogen sequence identity: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

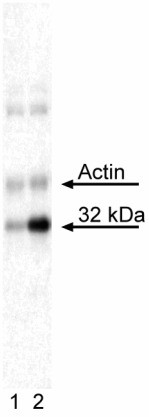
Description

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 N5-676 monoclonal antibody specifically detects the S6 ribosomal protein phosphorylated at S244.



Analysis of S6 (pS244) 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 (pS244). 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 (pS244). *The specificity of mAb N5-676 was confirmed by western blot analysis using unconjugated Mouse anti-S6 (pS244) antibody on lysates from untreated (lane 1) or PMA-treated (lane 2) PBMC. S6 (pS244) is identified as a band of 32 kDa, with increased intensity in the PMA-treated cells. Quantitative western blot analyses demonstrated that the unidentified high-molecular-weight bands are not induced by the PMA treatment (data not shown) and, thus, do not contribute to the signal increase that is observed by flow cytometry. Purified Mouse anti-Actin monoclonal antibody (Cat. No. 612656 or 612657) was the gel-loading control.*

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Preparation and Storage

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

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	PHA-stimulated PBMC	PMA	Cytofix	Perm I, II, or III	Upregulated expression
	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 + S244 phospho peptide			32-kDa band decreased
	Human	HEK 293	20% FBS + S244 non-phospho peptide			32-kDa band not affected
	Human	HEK 293	20% FBS + S240 or S235/S236 phospho peptide			32-kDa band not affected
	Human	PHA-stimulated PBMC	Untreated			32- and 94-kDa bands
	Human	PHA-stimulated PBMC	PMA			32-kDa band increased
	Human	PBMC	Untreated			32- and 94-kDa bands
	Human	PBMC	PMA			32-kDa band increased

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Bioimaging	Not Recommended

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human peripheral blood mononuclear cells using BD Cytofix™ Fixation Buffer. Any of the three BD™ Phosflow permeabilization buffers may be used.

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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. 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.
6. 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.
7. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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