

Technical Data Sheet

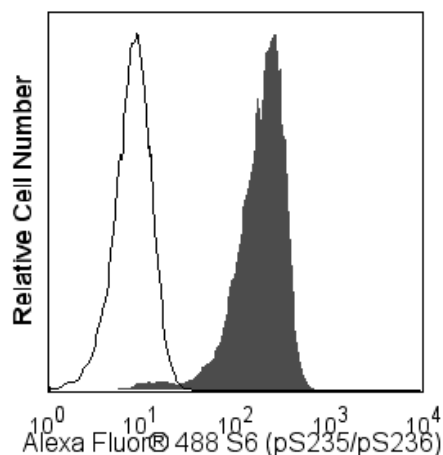
Alexa Fluor® 488 Mouse anti-S6 (pS235/pS236)**Product Information**

Material Number:	560434
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.

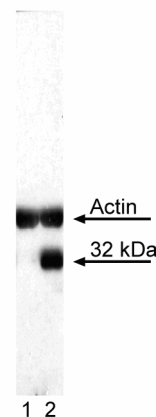
Description

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/10⁶ cells for 30 minutes (shaded histogram). Cells were then fixed in BD Cytotfix™ 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® 488 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.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	800.979.9408	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit bdbiosciences.com/contact

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

Unless otherwise noted, BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2011 BD



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® 488 under optimum conditions, and unreacted Alexa Fluor® 488 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	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- μ l 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
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

- Blatt K, Herrmann H, Mirkina I, et al. The PI3-kinase/mTOR-targeting drug NVP-BE2235 inhibits growth and IgE-dependent activation of human mast cells and basophils. *PLoS ONE*. 2012; 7(1):e29925. (Clone-specific: Flow cytometry)
- Corradetti MN, Inoki K, Guan KL. The stress-induced proteins RTP801 and RTP801L are negative regulators of the mammalian target of rapamycin pathway. *J Biol Chem*. 2005; 280(11):9769-9772. (Biology)
- Gu JJ, Santiago L, Mitchell BS. Synergy between imatinib and mycophenolic acid in inducing apoptosis in cell lines expressing Bcr-Abl. *Blood*. 2005; 105(8):3270-3277. (Biology)
- Jastrzebski K, Hannan KM, Tchoubrieva EB, Hannan RD, Pearson RB. Coordinate regulation of ribosome biogenesis and function by the ribosomal protein S6 kinase, a key mediator of mTOR function. *Growth Factors*. 2007; 25(4):209-226. (Biology)
- Kleijn M, Proud CG. The regulation of protein synthesis and translation factors by CD3 and CD28 in human primary T lymphocytes. *BMC Biochem*. 2002; 3:11. (Biology)
- Lai L, Li Y, Smith J, et al. Activation of the p70 S6 kinase by all-trans-retinoic acid in acute promyelocytic leukemia cells. *Blood*. 2005; 105(4):1669-1677. (Biology)
- Shah OJ, Ghosh S, Hunter T. Mitotic regulation of ribosomal S6 kinase 1 involves Ser/Thr, Pro phosphorylation of consensus and non-consensus sites by Cdc2. *J Biol Chem*. 2003; 278(18):16433-16442. (Biology)
- Shah OJ, Hunter T. Critical role of T-loop and H-motif phosphorylation in the regulation of S6 kinase 1 by the tuberous sclerosis complex. *J Biol Chem*. 2004; 279(20):20816-20823. (Biology)
- van de Laar L, van den Bosch A, Boonstra A, et al. PI3K-PKB hyperactivation augments human plasmacytoid dendritic cell development and function. *Blood*. 2012; 120(25):4982-4991. (Clone-specific: Flow cytometry)

BD Biosciences

bdbiosciences.com

United States 877.232.8995 Canada 800.979.9408 Europe 32.53.720.550 Japan 0120.8555.90 Asia Pacific 65.6861.0633 Latin America/Caribbean 55.11.5185.9995

For country contact information, visit bdbiosciences.com/contact

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

Unless otherwise noted, BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2011 BD

