**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.

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**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® 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.
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.

### Application Notes

#### Application

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<thead>
<tr>
<th>Application</th>
<th>Routine Tested</th>
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<tbody>
<tr>
<td>Intracellular staining</td>
<td>Routinely Tested</td>
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<tr>
<td>Bioimaging</td>
<td>Not Recommended</td>
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</tbody>
</table>

#### 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

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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</thead>
<tbody>
<tr>
<td>554655</td>
<td>Fixation Buffer</td>
<td>100 ml</td>
<td>(none)</td>
</tr>
<tr>
<td>557885</td>
<td>Perm/Wash Buffer I</td>
<td>125 ml</td>
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</tr>
<tr>
<td>558052</td>
<td>Perm Buffer II</td>
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</tr>
<tr>
<td>558050</td>
<td>Perm Buffer III</td>
<td>125 ml</td>
<td>(none)</td>
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#### 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. 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.

#### References