Technical Data Sheet

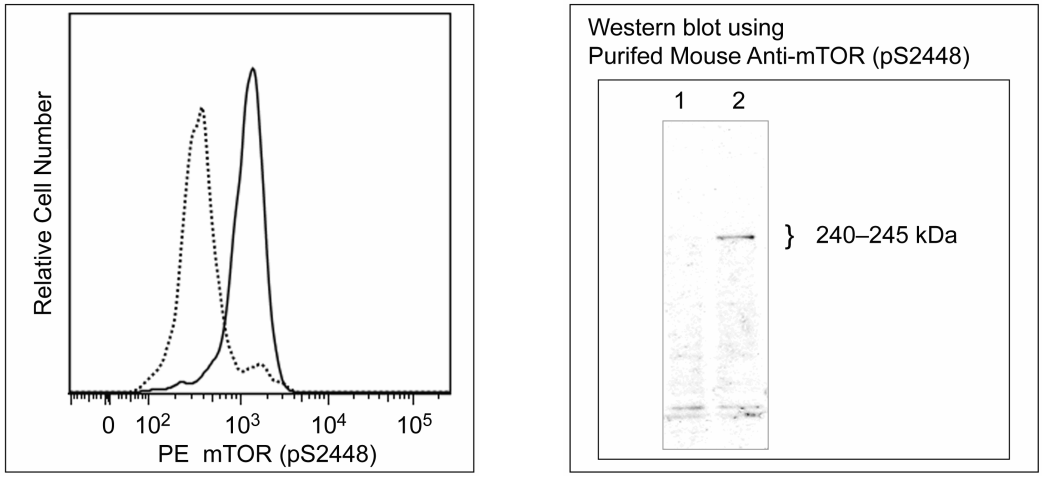
PE Mouse Anti-mTOR (pS2448)

Product Information

Material Number:	**563489**
Alternate Name:	Mammalian target of rapamycin; FRAP1; RAFT1; RAPT1
Size:	50 tests
Vol. per Test:	5 µl
Clone:	O21-404
Immunogen:	Phosphorylated Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Predicted Reactivity: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The O21-404 monoclonal antibody recognizes the human mammalian target of rapmycin,(mTOR) that is phosphorylated at serine residue 2448, mTOR (pS2448). mTOR belongs to the phosphoinositide-3-kinase (PI3K)-related (PIKK) family of kinases. mTOR is also known as mechanistic target of rapamycin (serine/threonine kinase), FRAP, RAFT1, and RAPT1. mTOR functions as an amino acid and ATP sensor to balance nutrient availability and cell growth. When nutrients are sufficiently available, mTOR is activated by phosphorylation at serine residue 2448 through the PI3 kinase/Akt signaling pathway. Phosphorylated mTOR in turn activates the p70 S6 kinase and contributes to the inactivation 4E-BP1, an elF4E inhibitor. In this way, mTOR participates in the upregulation of cellular protein synthesis, growth, proliferation and survival. mTOR function may be abnormally regulated in tumors.



Analyses of mTOR (pS2448) expression in stimulated human peripheral blood B cells.

Left Panel: Flow cytometric analysis of mTOR (pS2448) expression. *Human B lymphocytes were prepared from peripheral blood mononuclear cells (PBMC) by negative selection using the BD IMag™ Human B Lymphocyte Enrichment Set - DM (Cat. No. 558007). The B cells were serum starved overnight in culture and were either not stimulated (dashed line histogram) or were stimulated with Type C CpG oligonucleotide (1 µM, 4 hrs; InvivoGen, Cat. No. TLRL-2395). The cells were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655; 10 minutes at 37°C) and then permeabilized by adding BD Phosflow™ Perm Buffer III (Cat. No. 558050; 30 minutes on ice). The cells were washed twice with BD Pharmingen™ Stain Buffer (Cat. No. 554656) and then stained with BD Phosflow™ PE Mouse Anti-mTOR (pS2448) (Cat. No. 563489) and Alexa Fluor® 647 Mouse Anti-Human CD20 (Cat. No. 558054) antibodies using the BD Biosciences Protocol for Intracellular Staining. The fluorescence histograms were derived from CD20 positive-gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometric analysis was performed using a BD FACSCanto™ II Flow Cytometer.*

Right Panel: Western blot analysis of mTOR (pS2448) expression. *Aliquots of the unstimulated (lane 1) or CPG-stimulated (lane 2) B cells described above were made into lysates. The lysates were electrophoresed, transferred to membranes and blotted using Purified Mouse Anti-mTOR (pS2448) antibody (Clone O21-404; 2 µg/mL). Phosphorylated mTOR (pS2448) was identified as ~240-245 kDa band.*

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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 with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
558007	Human B Lymphocyte Enrichment Set - DM	5.0 ml	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
558054	Alexa Fluor® 647 Mouse Anti-Human CD20	50 tests	H1

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. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
3. 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.
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References

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Chiu MI, Katz H, Berlin V. RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. *Proc Natl Acad Sci U S A.* 1994; 91(26):12574-12578. (Biology)

Holz MK, Blenis J. Identification of S6 kinase 1 as a novel mammalian target of rapamycin (mTOR)-phosphorylating kinase. *J Biol Chem.* 2005; 280(28):26089-26093. (Biology)

Johnson SC, Rabinovitch PS, Kaeberlein M. mTOR is a key modulator of ageing and age-related disease. *Nature.* 2013; 493(7432):338-345. (Biology)

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Sengupta S, Peterson TR, Sabatini DM. Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell.* 2010; 40(2):310-322. (Biology)

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