Store at 4°C

#5123

Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (Alexa Fluor® 647 Conjugate)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez-Gene ID #1978
Swiss-Prot Acc. #Q13541

Applications	Species Cross-Reactivity*	Isotype
F Endogenous	H, M, R, Mk	Rabbit IgG

Description: This Cell Signaling Technology (CST) antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated antibody, Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb #2855, reacts with Phospho-4E-BP1 (Thr37/46) from human, mouse, rat and monkey. CST expects that phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (Alexa Fluor® 647 Conjugate) will also recognize Phospho-4E-BP1 in these species.

Background: Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the eIF4E translation initiation factor. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity (2,3). Multiple 4E-BP1 residues are phosphorylated *in vivo* (4). While phosphorylation by FRAP/mTOR on Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70 (5).

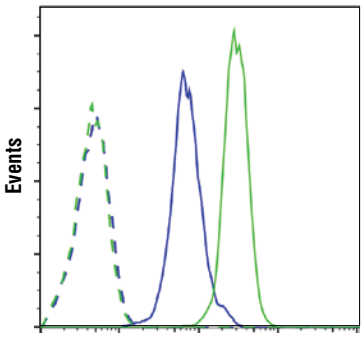
Specificity/Sensitivity: Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (Alexa Fluor® 647 Conjugate) detects endogenous levels of 4E-BP1 only when phosphorylated at Thr37 and/or Thr46. This antibody may cross-react with 4E-BP2 and 4E-BP3 when phosphorylated at equivalent sites.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr37 and Thr46 of mouse 4E-BP1. The antibody was conjugated to Alexa Fluor® 647 under optimal conditions with an F/P ratio of 2-5.

The Alexa Fluor® 647 dye is maximally excited by red light (e.g. 633 nm He-Ne laser). Antibody conjugates of the Alexa Fluor® 647 dye produce bright far-red-fluorescence emission with a peak at 665 nm.

Background References:

(1) Pause, A. et al. (1994) *Nature* 371, 762–767.
(2) Brunn, G.J. et al. (1997) *Science* 277, 99–101.
(3) Gingras, A.C. et al. (1998) *Genes Dev.* 12, 502–513.
(4) Fadden, P. et al. (1997) *J. Biol. Chem.* 272, 10240–10247.
(5) Gingras, A.C. et al. (1999) *Genes Dev.* 13, 1422–1437.



Phospho-4E-BP1 (Thr37/46) (Alexa Fluor® 647 Conjugate)

Flow cytometric analysis of jurkat cells, untreated (green) or treated with LY294002 #9901 (50uM, 2hr), Wortmannin #9951 (1uM, 2hr), and U0126 #9903 (10uM, 2hr) (blue), using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (Alexa Fluor® 647 Conjugate) (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 647 Conjugate) #2985 (dashed lines).

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide, 2 mg/ml BSA. Store at 4°C. *Protect from light. Do not freeze.*

***Species cross-reactivity other than human is determined by western blot using the unconjugated antibody.**

Recommended Antibody Dilutions:

Flow Cytometry	1:50

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected