

# Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (Alexa Fluor® 488 Conjugate)

✓ 500 µl  
(50 tests)



**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
**Web** ■ www.cellsignal.com

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This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

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

**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 cap-dependent translation is activated (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).

**Description:** Cell Signaling Technology antibody conjugated to Alexa Fluor® 488 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated antibody, #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® 488 Conjugate) will also recognize Phospho-4E-BP1 in these species.

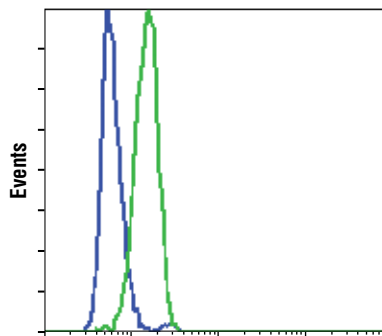
**Specificity/Sensitivity:** Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (Alexa Fluor® 488 Conjugate) detects endogenous levels of 4E-BP1 only when phosphorylated at Thr37 and/or Thr46. This antibody may cross-react with 4E-BP2 and 4E-BP3 when phosphorylated at equivalent sites.

**Source/Purification:** Monoclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Thr37 and Thr46 of mouse 4E-BP1. The antibody was conjugated to Alexa Fluor® 488 under optimal conditions with an F/P ratio of 2-5.

**Directions on Use:** Add 10 µl of the conjugated antibody to 500,000 cells in 90 µl PBS/0.5% BSA. See protocol for more details.

**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) Rabbit mAb (Alexa Fluor® 488 Conjugate)**

Flow cytometric analysis of Jurkat cells, untreated (green) or LY294002, Wortmannin and U0126-treated (blue), using Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (Alexa Fluor® 488 Conjugate).

**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 1:10

**Companion Products:**

- Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb #2855
- Phospho-4E-BP1 (Ser65) Antibody #9451
- Phospho-4E-BP1 (Thr70) Antibody #9455
- Phospho-4E-BP1 (Ser65) (174A9) Rabbit mAb #9456
- Nonphospho-4E-BP1 (Thr46) (87D12) Rabbit mAb #4923
- 4E-BP1 (53H11) Rabbit mAb #9644
- 4E-BP2 Antibody #2845
- Rabbit IgG Isotype Control (Alexa Fluor® 488 Conjugate) #4340

The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents. Alexa Fluor® is a registered trademark of Molecular Probes, Inc.

## Flow Cytometry Protocol for Intracellular Staining Using Conjugated Primary Antibodies

### A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g  $\text{Na}_2\text{HPO}_4$  and 0.24 g  $\text{KH}_2\text{PO}_4$  in 800 ml distilled water ( $\text{dH}_2\text{O}$ ). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100ml 1X PBS. Store at 4°C

### B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

### C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

### D Staining Using Conjugated Primary Antibodies

**NOTE:** Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot  $5 \times 10^5$  cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation.
3. Resuspend cells in 90  $\mu\text{l}$  Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10  $\mu\text{l}$  of conjugated antibody to the assay tubes.
6. Incubate for 30-60 minutes, in the dark at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.