

## Technical Data Sheet

## Alexa Fluor® 647 Mouse anti-4EBP1 (pT36/pT45)

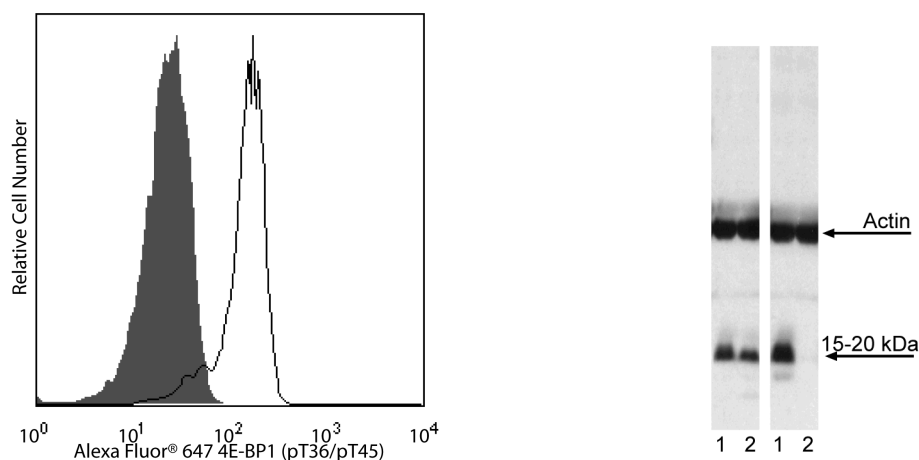
## Product Information

|                  |                                                                   |
|------------------|-------------------------------------------------------------------|
| Material Number: | 560286                                                            |
| Alternate Name:  | 4E-BP1, EIF4EBP1, P/OKCL6, PHAS-I, PHAS-1                         |
| Size:            | 50 tests                                                          |
| Vol. per Test:   | 20 µl                                                             |
| Clone:           | M31-16                                                            |
| Immunogen:       | Phosphorylated Human 4EBP1 (pT45) Peptide                         |
| Isotype:         | Mouse (BALB/c) IgG1, κ                                            |
| Reactivity:      | QC testing: Human<br>Predicted: Mouse, Rat                        |
| Storage Buffer:  | Aqueous buffered solution containing BSA and ≤0.09% sodium azide. |

## Description

The eukaryotic translation initiation factor **4E-Binding Protein 1** (4EBP1) is a phosphorylated heat- and acid-stable protein (PHAS-I or PHAS-1), and it is regulated by insulin. It is a member of the eIF4E-Binding Protein Family, which also includes the proteins 4EBP2 and 4EBP3. 4EBP1 binds with eukaryotic translation Initiation Factor **4E** (eIF4E), which prevents its assembly into the eIF4E complex and inhibits cap-dependent translation. When 4EBP1 is phosphorylated, this binding is disrupted, allowing cap-dependent translation to be activated. Phosphorylation of 4EBP1 is required for protein synthesis, and it mediates the regulation of protein translation by stimuli that signal through the phosphoinositide 3 (PI3) kinase pathway. We found that threonines 36 and 45 (T36/T45) are phosphorylated in resting human peripheral blood mononuclear cells. PI3 kinase inhibitors, such as LY294002 down-regulate the phosphorylation level of 4EBP1 (pT36/pT45).

The M31-16 monoclonal antibody recognizes the phosphorylated T36 and T45 of activated human 4EBP1. The orthologous phosphorylation sites in mouse and rat 4EBP1 are T35 and T44.



**LEFT PANEL:** Analysis of 4EBP1 (pT36/pT45) in human peripheral blood monocytes. Human peripheral blood mononuclear cells (PBMC) were either treated with 100 µM LY294002 (Sigma, Cat. No. L-9908) for 1 hour at 37°C (shaded histogram) or untreated (open histogram). The PBMC were fixed (BD Cytotfix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, permeabilized with BD™ Phosflow Perm Buffer III (Cat. No. 558050) on ice for 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-4EBP1 (pT36/pT45). For data analysis, monocytes were selected by their scatter profile. The data demonstrates that the level of phosphorylation of 4EBP1 decreases when protein kinase activity is inhibited by the treatment. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.

**RIGHT PANEL:** The specificity of mAb M31-16 was confirmed by western blot analysis using unconjugated polyclonal anti-4EBP1 (Cell Signaling Technology, Cat. No. 9542, left blot) and unconjugated monoclonal Mouse anti-4EBP1 (pT36/pT45) (right blot) antibodies on lysates from control (lanes 1) and LY294002-treated (lanes 2) PBMC. 4EBP1 is identified as a band of 15-20 kDa in the left blot, regardless of LY294002 treatment. The right blot demonstrates the reduction of 4EBP1 (pT36/pT45) with LY294002 treatment (lane 2). 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  | 888.268.5430 | 32.53.720.550 | 0120.8555.90 | 65.6861.0633 | 0800.771.7157           |

For country-specific contact information, visit [bdbiosciences.com/how\\_to\\_order/](http://bdbiosciences.com/how_to_order/)

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.

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



## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

*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    | Untreated                                         | Cytofix or Fix I | Perm I, II, or III | Positive expression on lymphocytes & monocytes |
|        | Human   | PBMC    | Wortmannin &/or LY294002 kinase inhibitors        | Cytofix or Fix I | Perm I, II, or III | Down-regulation                                |
|        | Human   | PBMC    | Rapamycin                                         | Cytofix or Fix I | Perm III           | No change                                      |
| WB     | Human   | HEK 293 | Serum starvation                                  |                  |                    | 15-20-kDa band                                 |
|        | Human   | HEK 293 | Wortmannin                                        |                  |                    | 15-20-kDa band decreased                       |
|        | Human   | HEK 293 | 20% FBS                                           |                  |                    | 15-20-kDa band increased                       |
|        | Human   | HEK 293 | T36 or T45 phospho peptide                        |                  |                    | 15-20-kDa band decreased                       |
|        | Human   | HEK 293 | T64 or T69 phospho peptide or non-phospho peptide |                  |                    | 15-20-kDa band not affected                    |
|        | Human   | PBMC    | Untreated                                         |                  |                    | 15-20-kDa band                                 |
|        | Human   | PBMC    | CD3/CD28 crosslinking                             |                  |                    | 15-20-kDa band increased                       |
|        | Human   | PBMC    | LY294002 kinase inhibitor                         |                  |                    | 15-20-kDa band decreased                       |

## Application Notes

### Application

Intracellular staining (flow cytometry)

Routinely Tested

### Recommended Assay Procedure:

Either BD Cytofix™ fixation buffer or BD™ Phosflow Fix Buffer I may be used for cell fixation. 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) |
| 557870         | Fix Buffer I       | 250 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  $1 \times 10^6$  cells in a 100-μl experimental sample (a test).
2. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
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. 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.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.

## References

Gingras AC, Raught B, Gygi SP, et al. Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes Dev.* 2001; 15(21):2852-2864. (Biology)  
Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev.* 2004; 18:1926-1945. (Biology)