Technical Data Sheet

Alexa Fluor® 647 Mouse anti-4EBP1 (pT69)

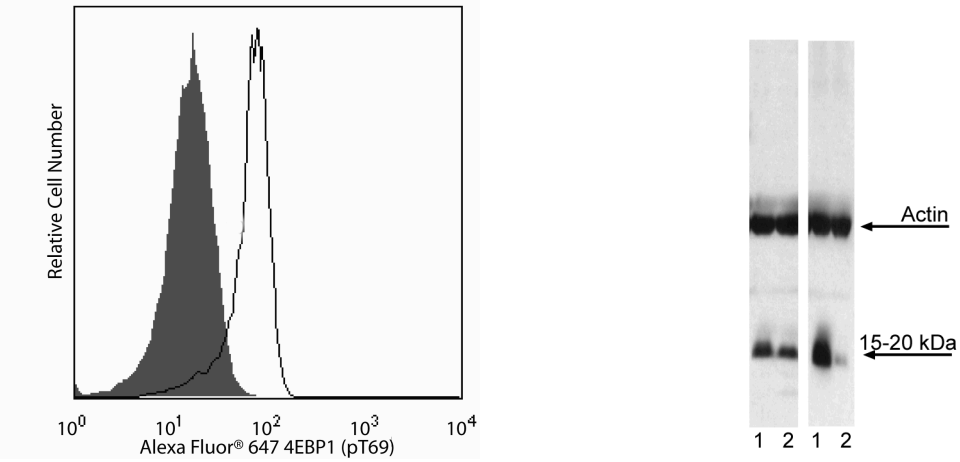
Product Information

Material Number:	**560289**
Alternate Name:	4E-BP1. EIF4EBP1, P/OKCL.6, PHAS-I, PHAS-1
Size:	50 tests
Vol. per Test:	20 µl
Clone:	M34-273
Immunogen:	Phosphorylated Human 4EBP1 (pT69) Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The eukaryotic translation initiation factor **4E**-**B**inding **P**rotein **1** (4EBP1) is a **p**hosphorylated **h**eat- and **a**cid-*s*table 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 **e**ukaryotic translation **I**nitiation **F**actor **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 threonine 69 (T69) is phosphorylated in resting human peripheral blood monocytes, but it is almost undetectable in resting lymphocytes. PI3 kinase inhibitors, such as LY294002 down-regulate the phosphorylation level of 4EBP1 (pT69) in monocytes.

The M34-273 monoclonal antibody recognizes the phosphorylated T69 of activated human 4EBP1.



LEFT PANEL: Analysis of 4EBP1 (pT69) 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 37ºC (shaded histogram) or untreated (open histogram). The PBMC were fixed (BD Cytofix™ 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 (pT69). 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 M34-273 was confirmed by western blot analysis (right panel) using unconjugated polyclonal anti-4EBP1 (Cell Signaling Technology, Cat. No. 9542, left blot) and unconjugated monoclonal Mouse anti-4EBP1 (pT69) (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 (pT69) with LY294002 treatment (lane 2). Purified Mouse anti-Actin monoclonal antibody (Cat. No. 612656 or 612657) was the gel-loading control.*

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.

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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 in monocytes, but not in lymphocytes
	Human	PBMC	Wortmannin &/or LY294002 kinase inhibitors	Cytofix or Fix I	Perm I, II, or III	Down-regulation in monocytes
	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	T69 phospho peptide			15-20-kDa band decreased
	Human	HEK 293	T36, T45, or T64 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 not affected
	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 × 10^6 cells in a 100-µl experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharmingen/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.
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)