Technical Data Sheet

PE Mouse anti-eIF4E (pS209)

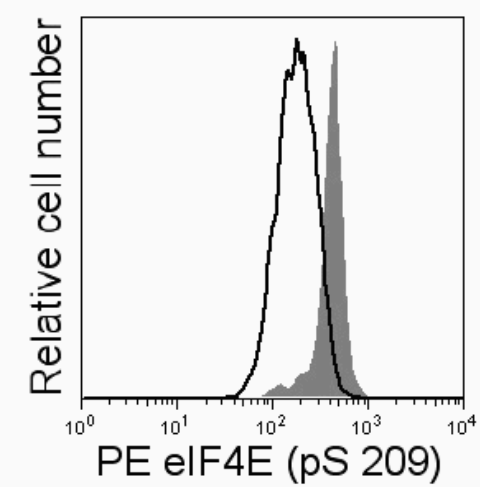
Product Information

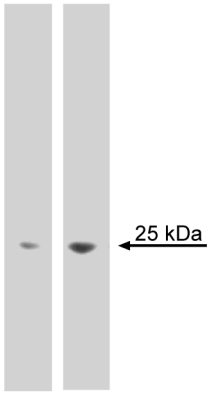
Material Number:	**560229**
Alternate Name:	eIF-4E, CBP, EIF4E1, EIF4EL1, EIF4F, mRNA cap-binding protein
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J77-925
Immunogen:	Phosphorylated Human eIF4E Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Predicted: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The *e*ukaryotic translation *I*nitiation *F*actor *4E* (eIF4E) is a 25-kDa phosphoprotein that specifically binds to the 7-methylguanosine-containing cap of mRNA. eIF4E is the rate-limiting component for the initiation of cap-dependent translation by the eIF4F translation initiation complex, which is composed of eIF4E, eIF4G, and eIF4A. This complex promotes the unwinding of secondary structure at the 5' untranslated region of mRNA that is necessary to expose and locate the AUG initiation codon. Other functions of eIF4E have been identified, such as promoting the export of mRNAs that are involved in cell cycle progression from the nucleus and differentially regulating the translation of certain mRNAs in the cytoplasm. Three mechanisms for eIF4E regulation have been identified: Mnk1-mediated phosphorylation on serine 209 (S209) is required for eIF4E binding to the cap structure; over-expression of phosphorylated eIF4E can lead to increased cell proliferation, suppression of apoptosis, and a transformed phenotype; and interactions with nonphosphorylated eIF4E-binding proteins inhibit the formation of the eIF4F complex.

The J77-925 monoclonal antibody recognizes the phosphorylated S209 (pS209) of eIF4E.





Analysis of eIF4E (pS209) in monocytes. *Human peripheral blood mononuclear cells (PBMC) were either stimulated with 40 nM Phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich Cat. No. P8139) for 15 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes, then permeabilized (BD™ Phosflow Perm Buffer II, Cat. No. 558052) on ice for at least 30 minutes, and then stained with PE Mouse anti-eIF4E (pS209). Monocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSArray™ bioanalyzer system.*

The specificity of mAb J77-925 was confirmed by western blot analysis using unconjugated antibody on lysates from control (lane 1) and PMA-treated (lane 2) PBMC. eIF4E (pS209) is identified as a band of 25 kDa, with increased intensity in the treated cells.

Preparation and Storage

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.
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	HeLa S3	Anisomycin	Cytofix	Perm III	Unsatisfactory
	Human	HeLa S3	λ or alkaline phosphatase	Cytofix	Perm III	Unsatisfactory
	Human	Jurkat	PMA	Cytofix	Perm III	Induction observed
	Human	Whole Blood	PMA	Lyse/Fix	Perm III	Unsatisfactory
	Human	PBMC	Anisomycin	Cytofix	Perm III	Unsatisfactory
	Human	PBMC	PMA	Lyse/Fix	Perm III	Unsatisfactory
	Human	PBMC	PMA	Cytofix	Perm I, II, or III	Greater induction on monocytes than lymphocytes, Perms I & II better than Perm III
WB	Human	HeLa S3	Anisomycin			25-kDa band induced
	Human	HeLa S3	Anisomycin + non-phopho peptide or alkaline phosphatase			No blocking of 25-kDa band
	Human	HeLa S3	Anisomycin + phopho peptide or λ phosphatase			Blocking of 25-kDa band
	Human	Jurkat or PBMC	PMA			25-kDa band induced

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. 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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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Grolleau A, Kaplan MJ, Hanash SM, Beretta L, Richardson B. Impaired translational response and increased protein kinase PKR expression in T cells from lupus patients. *J Clin Invest.* 2000; 106(12):1561-1568. (Biology)

Mendez R, Myers Jr MG, White MF, Rhoads RE. Stimulation of protein synthesis, eukaryotic translation initiation factor 4E phosphorylation, and PHAS-I phosphorylation by insulin requires insulin receptor substrate 1 and phosphatidylinositol 3-kinase. *Mol Cell Biol.* 1996; 16(6):2857-2864. (Biology)

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