Technical Data Sheet

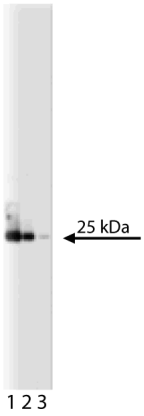
Purified Mouse Anti- eIF-4E

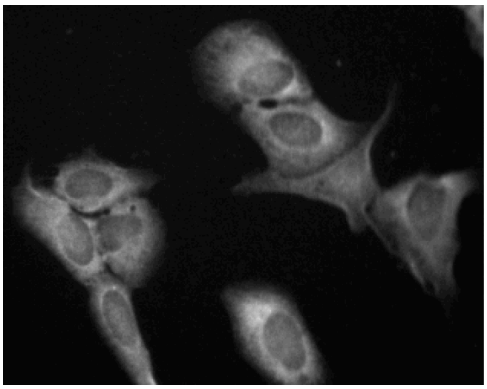
Product Information

Material Number:	**610270**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	87/eIF-4E
Immunogen:	Rabbit eIF-4E aa. 1-217
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Frog, Mouse, Rat
Target MW:	25 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The eukaryotic translation initiation factor 4E (eIF-4E) is a 25 kDa phosphoprotein that specifically binds to the 7-methylguanosine-containing cap of mRNA. eIF-4E is the rate-limiting component for the initiation of cap-dependent translation by the eIF-4E translation initiation complex. This complex promotes the unwinding of secondary structure at the 5' untranslated region of mRNA, which is necessary to expose and locate the AUG-initiation codon. Phosphorylation of eIF-4E on Ser-209 occurs after serum treatment in CHO cells, and may regulate its function. Overexpression of eIF-4E can lead to increased cell proliferation, transformation, and tumorigenesis in nude mice. The overexpression of a Ala-53 variant of eIF-4E cannot evoke these changes, suggesting that Ser-53 on eIF-4E participates in the transfer of mRNA to the 48S initiation complexes. In cooperation with nuclear oncogenes such as *c-myc* or *E1A*, eIF-4E transforms primary cells. Other studies have demonstrated that overexpression of eIF-4E causes activation of Ras and leads to a transformed phenotype. Subsequent overexpression of GAP then causes reversion of this phenotype. The mechanism by which eIF-4E plays a role in transformation is not clear, but it is postulated that high levels of eIF-4E may lead to the translation of mRNAs that are normally translationally repressed.





Left: Western blot analysis of eIF-4E on a A431 lysate. *Lane 1: 1:500, lane 2: 1:1000, lane 3: 1:2000 dilution of the eIF-4E antibody.*
Right: Immunofluorescent staining of U-2 OS (ATCC HTB-96) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-eIF-4E antibody. The second step reagent was Alexa Fluor 488 goat anti mouse Ig (Invitrogen). Images were taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and HeLa (ATCC CCL-2) cells. The Triton™ X-100 perm protocol is not recommended for use with this antibody. (see Recommended Assay Procedure)*

Preparation and Storage

Store undiluted at -20°C.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611447	A431 Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

De Benedetti A, Rhoads RE. Overexpression of eukaryotic protein synthesis initiation factor 4E in HeLa cells results in aberrant growth and morphology. *Proc Natl Acad Sci U S A.* 1990; 87(21):8212-8216. (Biology)

Jiang YP, Ballou LM, Lin RZ. Rapamycin-insensitive regulation of 4e-BP1 in regenerating rat liver. *J Biol Chem.* 2001; 276(14):10943-10951. (Clone-specific: Western blot)

Rhoads RE. Regulation of eukaryotic protein synthesis by initiation factors. *J Biol Chem.* 1993; 268(5):3017-3020. (Biology)

Seki N, Takasu T, Mandai K, et al. Expression of eukaryotic initiation factor 4E in atypical adenomatous hyperplasia and adenocarcinoma of the human peripheral lung. *Clin Cancer Res.* 2002; 8(10):3046-3053. (Clone-specific: Immunohistochemistry, Western blot)

Tang SJ, Reis G, Kang H, Gingras AC, Sonenberg N, Schuman EM. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proc Natl Acad Sci U S A.* 2002; 99(1):467-472. (Clone-specific: Immunofluorescence)