

Technical Data Sheet

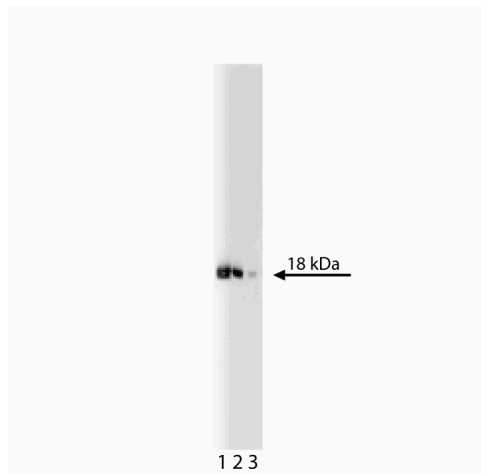
Purified Mouse Anti-eIF-5a

Product Information

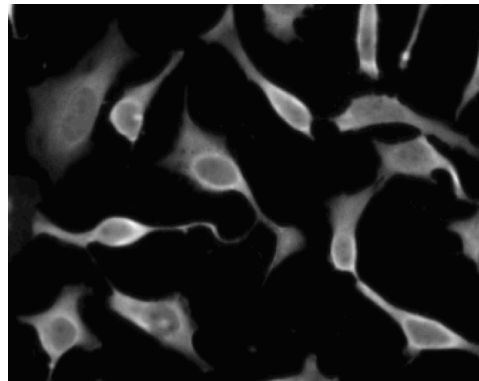
Material Number:	611977
Size:	150 µg
Concentration:	250 µg/ml
Clone:	26/eIF-5a
Immunogen:	Human eIF-5a aa. 58-154
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Mouse, Rat
Target MW:	18 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Initiation of eukaryotic translation involves a series of reactions mediated by multiple eukaryotic initiation factors (eIFs). However, one member of this family, eIF-5a, may have multiple protein functions. eIF-5a was originally identified as an initiation factor due to its binding of ribosomes, and its stimulation of methionyl-puromycin. Regulation of initiation may not be the major function of eIF-5a, since it functions as a targeting protein for the HIV protein, Rev, and the T-cell leukemia virus type 1 protein, Rex. In *Xenopus* oocytes, eIF-5a interacts with the nucleoporins Nup214, Nup153, Nup98, and Nup62, and is required for Rev-NES interaction with exportin1. These interactions may be involved with eIF-5a nuclear export of Rev protein, and eIF-5a requirement for Rev-mediated viral RNA export. eIF-5a is ubiquitously expressed, and is the only known eukaryotic protein that contains the post-translationally formed hypusine residue. Modification of eIF-5a with hypusine is coupled to cell proliferation, and disruption of this residue in yeast eIF-5a is lethal. Thus, eIF-5a may be an important nuclear transport protein with conserved function in many species.



Western blot analysis of eIF-5a on a Jurkat lysate.
Lane 1: 1:10000, lane 2: 1:20000, lane 3: 1:40000 dilution of the anti-eIF-5a antibody.



Immunofluorescent staining of HeLa (ATCC CCL-2) 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-5a antibody. The second step reagent was Alexa Fluor® 555 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 U-2 OS (ATCC HTB-96) cells and worked with both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).

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Preparation and Storage

Store undiluted at -20°C.

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

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

- 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.
- 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).
- Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
 - 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
 - Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
- Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
- 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.
- 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.
- Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
- 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.
- Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
- 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.
- 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/certified_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
611451	Jurkat Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(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

- Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- 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.
- 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.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- Triton is a trademark of the Dow Chemical Company.

References

Hofmann W, Reichart B, Ewald A, et al. Cofactor requirements for nuclear export of Rev response element (RRE)- and constitutive transport element (CTE) -containing retroviral RNAs. An unexpected role for actin. *J Cell Biol.* 2001; 152(5):895-910. (Biology)

Koettnitz K, Wohl T, Kappel B, Lottspeich F, Hauber J, Bevec D. Identification of a new member of the human eIF-5A gene family. *Gene.* 1995; 159(2):283-284. (Biology)

Xu A, Chen KY. Hypusine is required for a sequence-specific interaction of eukaryotic initiation factor 5A with postsystematic evolution of ligands by exponential enrichment RNA. *J Biol Chem.* 2001; 276(4):2555-2561. (Biology)