Technical Data Sheet

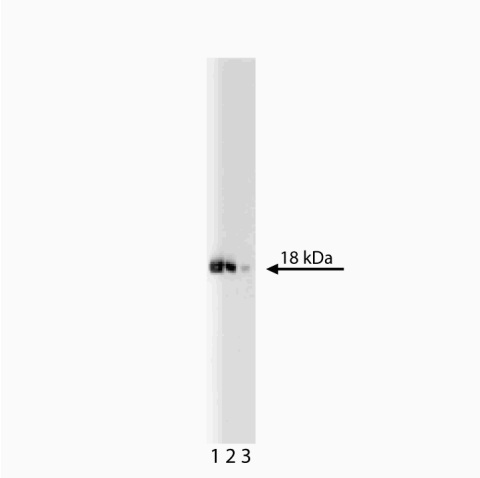
Purified Mouse Anti-eIF-5a

Product Information

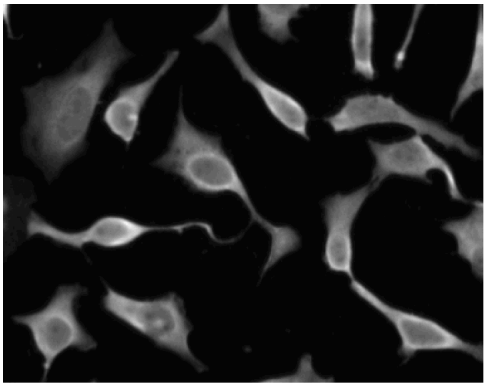
Material Number:	**611976**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	26/eIF-5a
Immunogen:	Human eIF-5a aa. 58-154
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Mouse, Rat
Target MW:	18 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, 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

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
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

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

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)