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Datasheet for ABIN968706 anti-EIF5A antibody (AA 58-154)

2 Images

2 Publications



Overview

Quantity:	50 µg
Target:	EIF5A
Binding Specificity:	AA 58-154
Reactivity:	Human, Mouse, Rat, Dog, Chicken
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This EIF5A antibody is un-conjugated
Application:	Western Blotting (WB), BioImaging (BI)

Product Details

Immunogen:	Human elF-5a aa. 58-154
Clone:	26-elF
Isotype:	lgG1
Cross-Reactivity:	Chicken, Dog (Canine), Mouse (Murine), Rat (Rattus)
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
	2. Please refer to us 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

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Product Details	
	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.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Target Details	
Target:	EIF5A

Alternative Name: eIF-5a (EIF5A Products)

Background: 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.

Molecular Weight:	18 kDa
Pathways:	Regulation of Muscle Cell Differentiation

Application Details

Application Notes:	Bioimaging
	1. Seed the cells in appropriate culture medium at \sim 10,000 cells per well in an 96-well Imaging
	Plate and culture overnight.
	2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation
	Buffer to each well. Incubate for 10 minutes at room temperature (RT).
	3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or
	Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes

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	at RT. OR b. Add 100 myl 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 myl of $1 \times PBS$.
	5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30
	minutes at RT.
	6. Remove the blocking buffer and add 50 myl 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 myl of $1 \times$ PBS.
	8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in
	50 myl 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 myl of $1 \times$ PBS.
	10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml
	Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging.
	11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN968537, ABIN967389
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	250 µg/mL
Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤ 0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Store undiluted at -20°C.

Publications

Product cited in: Xu, Chen: "Hypusine is required for a sequence-specific interaction of eukaryotic initiation factor
5A with postsystematic evolution of ligands by exponential enrichment RNA." in: The Journal of
biological chemistry, Vol. 276, Issue 4, pp. 2555-61, (2001) (PubMed).

Koettnitz, Wöhl, Kappel, Lottspeich, Hauber, Bevec: "Identification of a new member of the

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Images



Immunofluorescence

Image 1. Immunofluorescent staining of HeLa (ATCC CCL-2) cells. Cells were seeded in a 96 well imaging plate 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.

Western Blotting

Image 2. 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.



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