abcam

Product datasheet

Anti-eIF4A3 antibody ab32485

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Overview

Product name	Anti-eIF4A3 antibody
Description	Rabbit polyclonal to eIF4A3
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, IP, ICC/IF, WB
Species reactivity	**Reacts with:** Rat, Human **Predicted to work with:** Chicken, Cow, Xenopus laevis, Zebrafish, Cynomolgus monkey
Immunogen	Synthetic peptide corresponding to Human eIF4A3 aa 1-100. (Peptide available as **ab32484**)
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32485 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IP	★★☆☆☆ (1)	Use a concentration of 5 µg/ml.
ICC/IF	★★★★★ (2)	Use a concentration of 5 µg/ml.
WB	★★★★☆ (6)	Use a concentration of 1 µg/ml. Detects a band of approximately 47 kDa (predicted molecular weight: 47 kDa).

Target

Function

ATP-dependent RNA helicase. Component of a splicing-dependent multiprotein exon junction complex (EJC) deposited at splice junction on mRNAs. The EJC is a dynamic structure consisting of a few core proteins and several more peripheral nuclear and cytoplasmic associated factors that join the complex only transiently either during EJC assembly or during subsequent mRNA metabolism. Core components of the EJC, that remains bound to spliced mRNAs throughout all stages of mRNA metabolism, functions to mark the position of the exon-exon junction in the mature mRNA and thereby influences downstream processes of gene expression including mRNA splicing, nuclear mRNA export, subcellular mRNA localization, translation efficiency and nonsense-mediated mRNA decay (NMD). Constitutes at least part of the platform anchoring other EJC proteins to spliced mRNAs. Its RNA-dependent ATPase and RNA-helicase activities are induced by CASC3, but abolished in presence of the MAGOH/RBM8A heterodimer, thereby trapping the ATP-bound EJC core onto spliced mRNA in a stable conformation. The inhibition of ATPase activity by the MAGOH/RBM8A heterodimer increases the RNA-binding affinity of the EJC. Involved in translational enhancement of spliced mRNAs after formation of the 80S ribosome complex. Binds spliced mRNA in sequence-independent manner, 20-24 nucleotides upstream of mRNA exon-exon junctions. Shows higher affinity for single-stranded RNA in an ATP-bound core EJC complex than after the ATP is hydrolyzed.

Tissue specificity

Ubiquitously expressed.

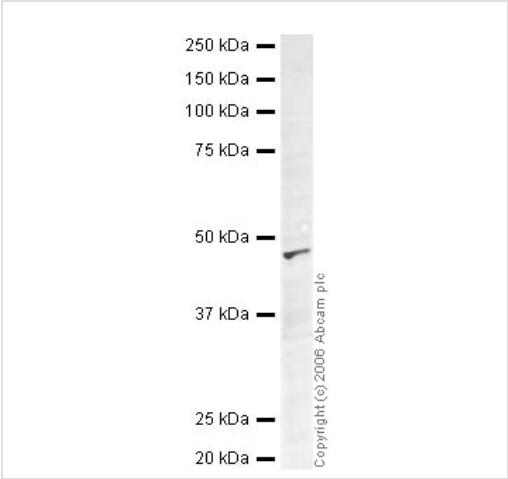
Sequence similarities

Belongs to the DEAD box helicase family. eIF4A subfamily.
Contains 1 helicase ATP-binding domain.
Contains 1 helicase C-terminal domain.

Cellular localization

Nucleus. Nucleus speckle. Cytoplasm. Nucleocytoplasmic shuttling protein. Travels to the cytoplasm as part of the exon junction complex (EJC) bound to mRNA. Detected in dendritic layer as well as the nuclear and cytoplasmic (somatic) compartments of neurons. Colocalizes with STAU1 and FMR1 in dendrites.

Images



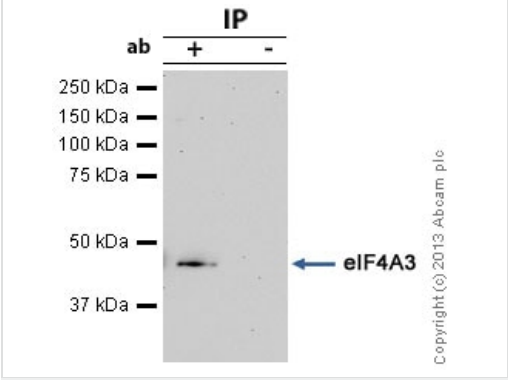
Western blot - Anti-eIF4A3 antibody (ab32485)

Anti-eIF4A3 antibody (ab32485) at 1 µg/ml + HeLa (Human epithelial carcinoma cell line) Nuclear Lysate at 20 µg

Secondary
IR Dye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/15000 dilution

Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 47 kDa



Immunoprecipitation - Anti-eIF4A3 antibody (ab32485)

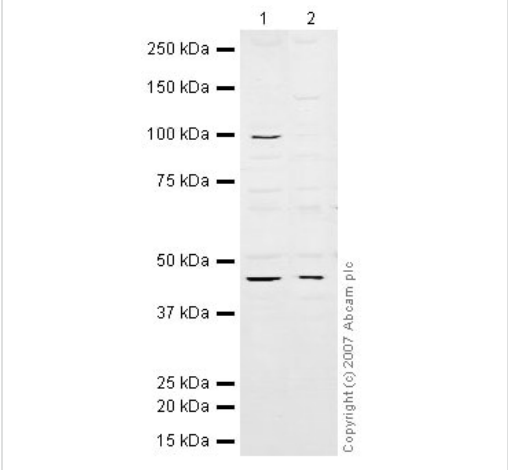
eIF4A3 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to eIF4A3 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab32485.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).

Band: 47kDa; eIF4A3



Western blot - Anti-eIF4A3 antibody (ab32485)

All lanes : Anti-eIF4A3 antibody (ab32485) at 1 µg/ml

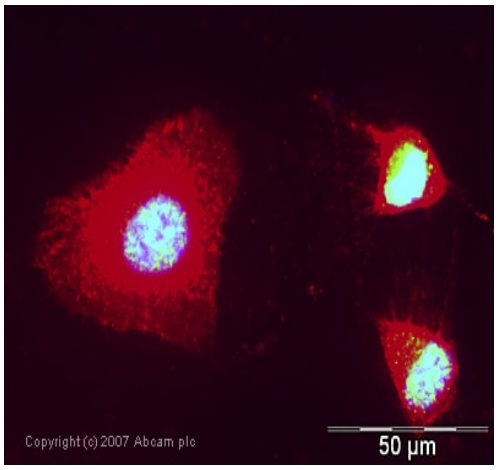
Lane 1 : MEF1 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
Lane 2 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

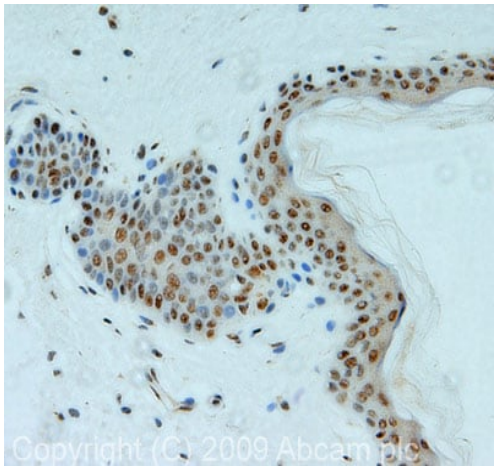
Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 46 kDa
Additional bands at: 100 kDa. We are unsure as to the identity of these extra bands.



Immunocytochemistry/ Immunofluorescence - Anti-eIF4A3 antibody (ab32485)

ICC/IF image of ab32485 stained human HeLa cells. The cells were PFA fixed (10 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab32485, 5µg/ml) for 1h at room temperature. 1%BSA / 10% normal serum / 0.3M glycine was used to quench autofluorescence and block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-eIF4A3 antibody (ab32485)

IHC image of eIF4A3 staining in human skin FFPE section, performed on a Leica Bond^{TM} system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab32485, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

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