abcam

ab57113

Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free

Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113) is a mouse monoclonal antibody provided in a PBS only buffer for easy conjugation detecting Argonaute-2 in **Western Blot, Flow Cytometry, IHC-P, ICC/IF**. Suitable for **Human,**.

- BSA, sodium azide, and glycerol-free for easy conjugation
- Over 160 publications
- Trusted since 2007

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human AGO2 aa 450 to C-terminus. The exact immunogen used to generate this antibody is proprietary information. Database link Q9UKV8
Carrier free	Yes
Clone number	2E12-1C9
Purification technique	Affinity purification Protein A
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Xenopus laevis
Dilution info	-
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

WB

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Xenopus laevis
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Xenopus laevis
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Xenopus laevis
Dilution info	-
Notes	-

Target data

See full target information AGO2

Function

Required for RNA-mediated gene silencing (RNAi) by the RNA-induced silencing complex (RISC). The 'minimal RISC' appears to include AGO2 bound to a short guide RNA such as a microRNA (miRNA) or short interfering RNA (siRNA). These guide RNAs direct RISC to complementary mRNAs that are targets for RISC-mediated gene silencing. The precise mechanism of gene silencing depends on the degree of complementarity between the miRNA or siRNA and its target. Binding of RISC to a perfectly complementary mRNA generally results in silencing due to endonucleolytic cleavage of the mRNA specifically by AGO2. Binding of RISC to a partially complementary mRNA results in silencing through inhibition of translation, and this is independent of endonuclease activity. May inhibit translation initiation by binding to the 7-methylguanosine cap, thereby preventing the recruitment of the translation initiation factor eIF4-E. May also inhibit translation initiation via interaction with EIF6, which itself binds to the 60S ribosomal subunit and prevents its association with the 40S ribosomal subunit. The inhibition of translational initiation leads to the accumulation of the affected mRNA in cytoplasmic processing bodies (P-bodies), where mRNA degradation may subsequently occur. In some cases RISC-mediated translational repression is also observed for miRNAs that perfectly match the 3' untranslated region (3'-UTR). Can also up-regulate the translation of specific mRNAs under certain growth

conditions. Binds to the AU element of the 3'-UTR of the TNF mRNA and up-regulates translation under conditions of serum starvation. Also required for transcriptional gene silencing (TGS), in which short RNAs known as antigene RNAs or agRNAs direct the transcriptional repression of complementary promoter regions. (Microbial infection) Upon Sars-CoV-2 infection, associates with viral miRNA-like small RNA, CoV2-miR-O7a, and may repress mRNAs, such as BATF2, to evade the IFN response.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113) is a mouse monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF) in Human, samples.

What is the molecular weight of Argonaute-2?
Anti-Argonaute-2 [2E12-1C9] - BSA and Azide free (ab57113) specifically detects a band for Argonaute-2 (UniProt: Q9UKV8) at a molecular weight of 97kDa.

Trusted by the scientific community
Anti-Argonaute-2 [2E12-1C9] - BSA and Azide free (ab57113) was first used in a scientific publication in 2007 and has been cited over 160 times in peer-reviewed journals.

Reviewed by scientists
Anti-Argonaute-2 [2E12-1C9] - BSA and Azide free (ab57113) has over 5 independent reviews from customers.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Argonaute-2 often referred to as Ago2 or Argonaute-2 protein plays an important role in the RNA-induced silencing complex (RISC). It has a molecular weight of approximately 97 kDa. Ago2 is found in the cytoplasm and nucleus of cells and is expressed in a wide range of tissues including the brain and liver. Its mechanical function involves binding to guide RNA and facilitating strand cleavage which is essential for gene silencing and regulation at the post-transcriptional level.
Biological function summary	The Argonaute-2 protein participates in gene silencing mechanisms. As a part of RISC Ago2 binds to microRNAs (miRNAs) or small interfering RNAs (siRNAs) to direct sequence-specific cleavage of target mRNAs regulating their translation and stability. This protein is a part of a multi-protein complex that modulates gene expression and influences cellular homeostasis and development. In its biological role Argonaute-2 impacts cellular differentiation proliferation and apoptosis.
Pathways	Several cellular processes require Argonaute-2 notably the RNA interference (RNAi) and microRNA (miRNA) pathways. In these pathways it interacts with Dicer a protein essential for the maturation of small RNA molecules and facilitates the gene silencing process. Ago2 is also involved in the regulation of gene expression during embryonic development and stress responses indicating its importance in maintaining cellular function and responding to environmental changes.
Associated diseases and disorders	Anomalies in Argonaute-2 activity correlate with cancer including lung and breast cancer where dysregulation of miRNA pathways can lead to unchecked cellular proliferation. It is also linked to neurological disorders like schizophrenia possibly due to its role in gene expression regulation in neural cells. Interactions between Ago2 and proteins like Dicer and other Argonautes suggest complex regulatory networks that when disrupted contribute to these diseases.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

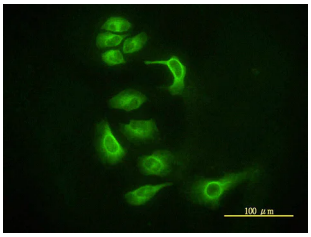
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

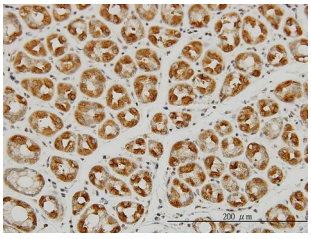
7 product images



Immunocytochemistry/ Immunofluorescence - Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113)

Argonaute-2 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Argonaute-2 antibody

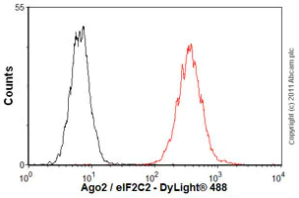
Ago2 / eIF2C2 antibody (ab57113) used in immunofluorescence at 10ug/ml on HeLa cells.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113)

Argonaute-2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Argonaute-2 antibody

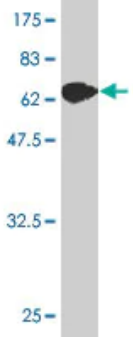
Ago2 / eIF2C2 antibody (ab57113) used in immunohistochemistry at 3ug/ml on formalin fixed and paraffin embedded human stomach.



Flow Cytometry - Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113)

Argonaute-2 Flow Cytometry staining using mouse Anti-Argonaute-2 antibody

Overlay histogram showing HeLa cells stained with ab57113 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab57113, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde/permeabilized in 0.1% PBS-Tween used under the same conditions.
This image was generated using the ascites version of the product.



Western blot - Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113)

Western Blot detection against the recombinant fragment imunogen (68 KDa for a.a. ~377 plus GST tag +26 kDa).
This image was generated using the ascites version of the product.

All lanes:
Western blot - Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113)

Predicted band size: 97 kDa

Observed band size: 68 kDa

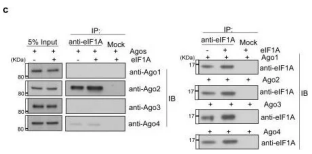


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Western blot - Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113)

Argonaute-2 Western blot staining using mouse Anti-Argonaute-2 antibody

Western Blotting using Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free, ab57113. Publication image from Gygi, S. P. et al., 2015, Nat Commun, 26018492. Legend direct from paper.

eIF1A interacts with Ago2(a) Proteins co-immunoprecipitated with Flag-HA-Ago2 from HEK293 cells were separated by SDS-PAGE (silver staining) and analyzed by mass spectrometry. eIF1A is a novel Ago2 interacting protein (*:detected peptides of eIF1A). (b) Fluorescence immunostaining was performed with anti-eIF1A (monoclonal) and anti-Ago2 (monoclonal) antibodies and DAPI (4′,6-diamidino-2-phenylindole) in HEK293 and human glioblastoma multiforme T98G cells. White arrows show the co-localized eIF1A and Ago2. (c) eIF1A interacts with Ago2. Human eIF1A was transiently overexpressed in stable HEK293 cells stably expressing Agos (1~4). Immunoprecipitation assays were performed with anti-eIF1A (monoclonal) antibody and immuno blotting (IB) with anti-Agos antibodies in the upper panel and anti-eIF1A antibodies in the bottom panel.

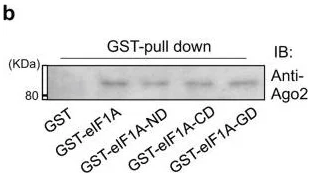


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Western blot - Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113)

Argonaute-2 Western blot staining using mouse Anti-Argonaute-2 antibody

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eIF1A binds to MID-domain of Ago2 in a RNA-binding-independent manner(a) Human eIF1A interacts with the MID fragment of human Ago2. Recombinantly expressed and Benzonase-treated Ago2 fragments and recombinantly expressed GST-eIF1A were used for GST-pull down analyses followed by Western blotting with anti-His antibodies (IB, immuno-blotting). The arrow shows that the MID fragment interacts with eIF1A. (b) The globular domain (GD) of eIF1A interacts with Ago2. Recombinantly expressed and Benzonase-treated GST, GST-eIF1A and mutants (ND, 25 ~ 144aa; CD, 1 ~ 114aa; GD, 25 ~ 114aa) were used for GST-pull down assays with cell lysates of HEK293 stably expressing Ago2. (c) 1H-15N TROSY-HSQC spectrum of 15N-eIF1A titrated with the sumo-MID (432 ~ 575aa). The titration of RNA-free-sumo-MID leads to broadening of RNA free 15N-eIF1A resonances (molar ratio of 1:1). Inserted frames show the broadening of resonances of V55, K56 and K67 residues in eIF1A upon MID titration. (d) 1H-15N TROSY-HSQC spectrum of 15N-Sumo-MID titrated with eIF1A. The titration of eIF1A (molar ratio of 1:1) results in broadening 1H-15N TROSY-HSQC crosspeaks coming from the 15N-MID domain, but not from 15N-Sumo (SMT3) tag. Inserted frames show the broadening of resonances of residues from MID-domain, but not from SMT3-Tag (SUMO-tag), upon eIF1A titration.

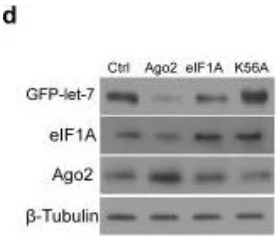


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Western blot - Anti-Argonaute-2 antibody [2E12-1C9] - BSA and Azide free (ab57113)

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eIF1A augments Ago2-mediated RNAi(a) Sequence of the human HMGA2 3′-UTR fragment mutant with two native let-7 target sites. Filled circles: Watson-Crick complementarity; open circles: non-Watson-Crick complementarity. Mutated nucleotides are shown in blue and wild type nucleotides were underlined (to neutralize site 2 and to reduce self-complementarity). (b) In vitro effects of eIF1A on Ago2-mediated RNA cleavage. eIF1A or the indicated mutants were incubated with Ago2, synthetic let-7a and 5′-32P end-labeled HMGA2 3′-UTR mutants. RNAs were analyzed by Northern blot and autoradiographs analyses. (c) Statistical analyses of three independent in vitro assays in (b). (d) Western blot assays of effects of eIF1A and eIF1A (K56A) mutant on GFP-let-7 reporter expression. Ago2, eIF1A or eIF1A (K56A) were transiently overexpressed in HEK293 cells stably expressing reporter GFP-let-7. (e)The expression ratio between Tubulin and GFP reporter in the same gel were calculated. Intact (100%) RISC activity was considered in control cells. The relative RISC activities under overexpression conditions of the indicated genes or mutants were obtained by comparison with the control. (f) Relative GFP reporter mRNAs in (d) by quantitative real-time PCR (qPCR). (g) Western blot assays of zebrafish c-Myb in the indicated zebrafish groups at 28 h.p.f (control, eif1axb MO (62.5μM, 1nl), eif1axb MO (62.5μM, 1nl) plus human eIF1A (heIF1A) (mRNA, 1μg/μl, 1000pl), and eif1axb MO (62.5μM, 1nl) plus heIF1A (K56A) mutant (mRNA, 1μg/μl, 1000pl)). Proteins were extracted from >10 zebrafish per group. (h) Relative c-Myb mRNAs in each group in (g) by qPCR. Total RNAs were extracted from >10 zebrafish per group. (i) Taqman PCR assays of relative zebrafish miR-126 (dre-miR-126) in each group in (g). Total RNAs were extracted from >10 zebrafish per group. In all statistical comparisons, three independent experiments were performed (mean ± s.d., n = 3 experiments, Student’ s t-test). *** reports p < 0.01.

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