Anti-Argonaute-2 antibody - ChIP Grade ab32381

Overview

Product name: Anti-Argonaute-2 antibody - ChIP Grade

Description: Rabbit polyclonal to Argonaute-2 - ChIP Grade

Host species: Rabbit

Tested applications: Suitable for: IP, IHC-P, ChIP, RIP, WB, ICC/IF

Species reactivity: Reacts with: Mouse, Rat, Human

Predicted to work with: Rabbit, Cow

Does not react with: Drosophila melanogaster

Immunogen: Synthetic peptide corresponding to Argonaute-2 aa 350-450 conjugated to keyhole limpet haemocyanin.

(Peptide available as ab32380)

Positive control: WB: HeLa whole cell lysate. IHC-P: Human breast cancer tissue.

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

Our Abpromise guarantee covers the use of ab32381 in the following tested applications.

- IP
- IHC-P
- ChIP
- RIP
- WB
- ICC/IF
Function

Required for RNA-mediated gene silencing (RNAi) by the RNA-induced silencing complex (RISC). The ‘minimal RISC’ appears to include EIF2C2/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 EIF2C2/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 upregulate the translation of specific mRNAs under certain growth conditions. Binds to the AU element of the 3'-UTR of the TNF (TNF-alpha) mRNA and upregulates 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.

Sequence similarities

Belongs to the argonaute family. Ago subfamily.
Contains 1 PAZ domain.
Contains 1 Piwi domain.

Domain

The Piwi domain may perform RNA cleavage by a mechanism similar to that of RNase H. However, while RNase H utilizes a triad of Asp-Asp-Glu (DDE) for metal ion coordination, this protein appears to utilize a triad of Asp-Asp-His (DDH).

Post-translational modifications

Hydroxylated. 4-hydroxylation appears to enhance protein stability but is not required for miRNA-binding or endonuclease activity.

Cellular localization

Cytoplasm > P-body. Nucleus. Translational repression of mRNAs results in their recruitment to P-bodies. Translocation to the nucleus requires IMP8.

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<th>Abreviews</th>
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<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 24280866</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>ChIP</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 19696410</td>
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<tr>
<td>RIP</td>
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<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 2 µg/ml. Detects a band of approximately 87 kDa (predicted molecular weight: 97 kDa).</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/200.</td>
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**Immunohistochemical analysis of Argonaute2 (AGO2) in human tissues sections from slowly progressive, rapidly progressive, or normal biopsies.**

Representative images of slowly progressive (A–B, E–F), rapidly progressive (C–D, G–H), and normal (I–J) biopsies stained with IgG control (A, C, E, G, & I) and anti-AGO2 antibody (B, D, F, H, & J) are shown. Images A, B, C, & D were stained with antibody concentrations of 20 µg/ml and images E, F, G, H, I, & J were stained with an antibody concentration of 4 µg/ml. Sections were counterstained with hematoxylin. Protein expression stains brown in this procedure (original magnification: ×200).

**Panels A-D shown.**

**All lanes**: Anti-Argonaute-2 antibody - ChIP Grade (ab32381) at 2 µg/ml

**Lane 1**: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

**Lane 2**: Jurkat whole cell lysate (ab7899)

**Lane 3**: HeLa (Human epithelial carcinoma cell line) Nuclear Lysate

Lysates/proteins at 20 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**: 97 kDa

**Observed band size**: 87 kDa

why is the actual band size different from the predicted?

**Additional bands at**: 55 kDa. We are unsure as to the identity of these extra bands.
The identification of the 55 kDa band is unclear but this band has also been observed in HeLa lysates in the Western Blot of ab5072, targeting Drosophila Ago2 / eIF2C2.

ab32381 (1/200) staining Ago2/ eIF2C2 in asynchronous HeLa (Human epithelial cell line from cervix adenocarcinoma) cells (green). Cells were fixed with paraformaldehyde, permeabilized with 0.5% TritonX100/ PBS and counterstained with DAPI in order to highlight the nucleus (red). Please refer to abreview for further experimental details.

IHC image of Ago2/eIF2C2 staining in human breast cancer FFPE section, performed on a Bond™ 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 ab32381, 5µg/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 hematoxylin and mounted with DPX.

RIP analysis of 368T1 murine lung cancer cells using ab32381 to bind Ago2 / eIF2C2.

Cells were transfected with an empty vector (empty), a vector expressing wild-type Hmga2 (wt), a vector expressing a let-7 site mutated Hmga2 (m7), a vector expressing wild-type Hmga2 without a start codon (ATG wt), or a vector expressing a let-7 site mutated Hmga2 without a start codon (ATG m7). RIP was performed with 5 µg Ago2 / eIF2C2 or IgG antibody per 10^7 cells. Binding was detected using qRT-PCR.

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