abcam

Product datasheet

Anti-Argonaute-2 antibody [EPR10410] ab156870

敲除验证 重组 RabMAb

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概述

产品名称	Anti-Argonaute-2抗体[EPR10410]
描述	兔单克隆抗体[EPR10410] to Argonaute-2
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human Argonaute-2 aa 1-100. The exact sequence is proprietary.
阳性对照	WB: HeLa, MCF7, HepG2 and K562 cell lysates. Rat liver and mouse liver lysates. IHC-P: Human breast carcinoma and human kidney tissues. Flow Cyt (intra): HeLa cells.
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
存储溶液	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR10410
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab156870于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/200.
WB		1/1000 - 1/10000. Predicted molecular weight: 97 kDa.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples. See **IHC antigen retrieval protocols**.
ICC/IF		1/100 - 1/250.
IP		Use at an assay dependent concentration.

靶标

功能

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.

序列相似性

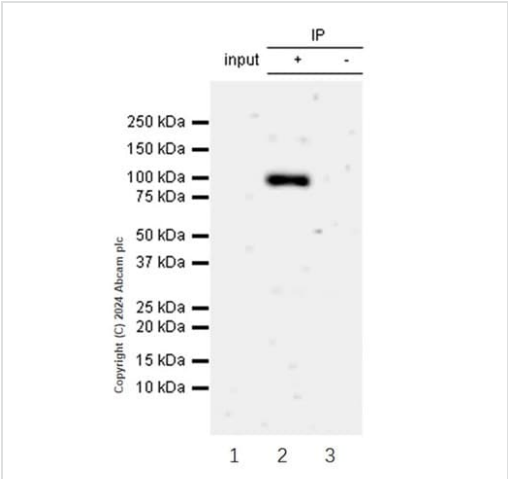
Belongs to the argonaute family. Ago subfamily.
Contains 1 PAZ domain.
Contains 1 Piwi domain.

结构域

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).

翻译后修饰	Hydroxylated. 4-hydroxylation appears to enhance protein stability but is not required for miRNA-binding or endonuclease activity.
细胞定位	Cytoplasm > P-body. Nucleus. Translational repression of mRNAs results in their recruitment to P-bodies. Translocation to the nucleus requires IMP8.

图片



Immunoprecipitation - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

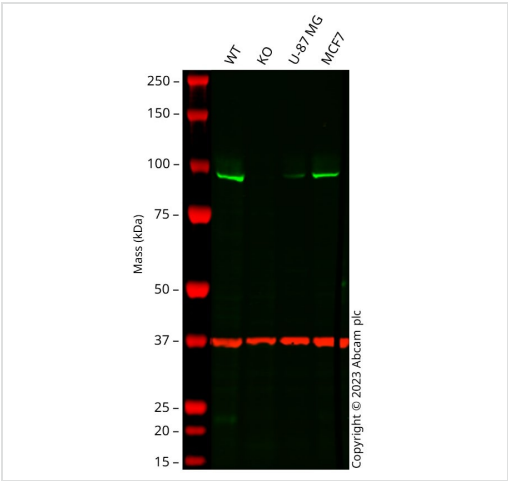
Argonaute-2 was immunoprecipitated from HeLa cell lysate with ab156870 at 1:30 dilution (2µg in 0.35mg lysates). Western blot was performed from the immunoprecipitate using ab156870 at 1/1000 dilution. Secondary antibody VeriBlot for IP secondary antibody (HRP) (**ab1313660**) was used at 1/5000 dilution.

Lane 1: HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate

Lane 2: HeLa (human cervical adenocarcinoma epithelial cell) whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab156870 in HeLa whole cell lysate

Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

All lanes : Anti-Argonaute-2 antibody [EPR10410] (ab156870) at 1/1000 dilution

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : AGO2 knockout HCT 116 cell lysate
Lane 3 : U-87 MG cell lysate
Lane 4 : MCF7 cell lysate

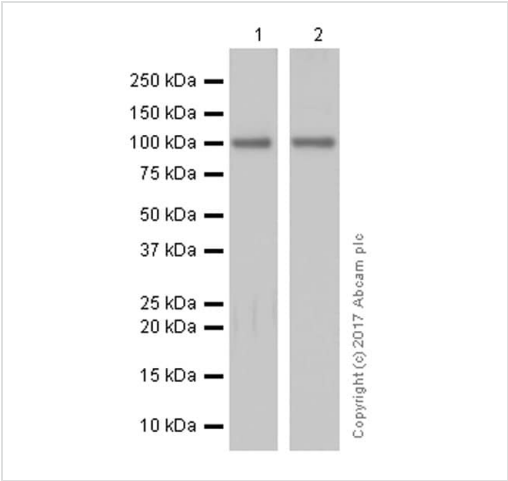
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 97 kDa
Observed band size: 95 kDa

False colour image of Western blot: Anti-Argonaute-2 antibody [EPR10410] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab156870 was shown to bind specifically to Argonaute-2. A band was observed at

95 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in AGO2 knockout cell line. To generate this image, wild-type and AGO2 knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

All lanes : Anti-Argonaute-2 antibody [EPR10410] (ab156870) at 1/5000 dilution (purified)

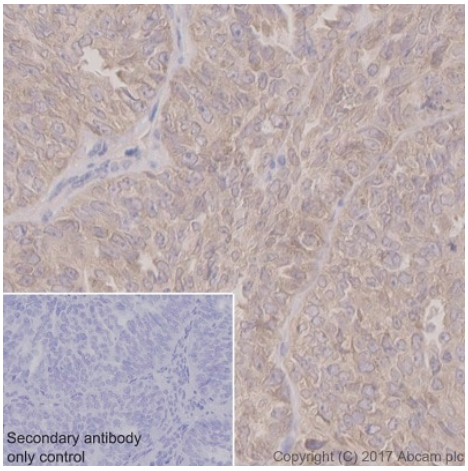
Lane 1 : Rat liver lysates
Lane 2 : Mouse kidney lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

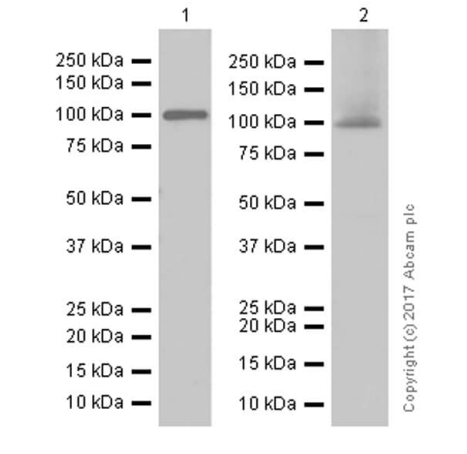
Predicted band size: 97 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human ovarian carcinoma tissue sections labeling Argonaute -2 with purified ab156870 at 1:100 dilution (1.9 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

All lanes : Anti-Argonaute-2 antibody [EPR10410] (ab156870) at 1/1000 dilution (purified)

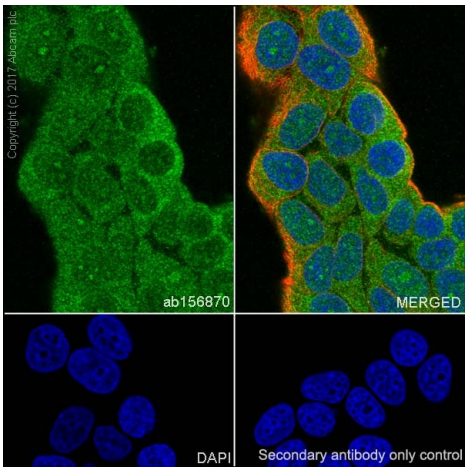
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HUVEC (Human umbilical vein endothelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

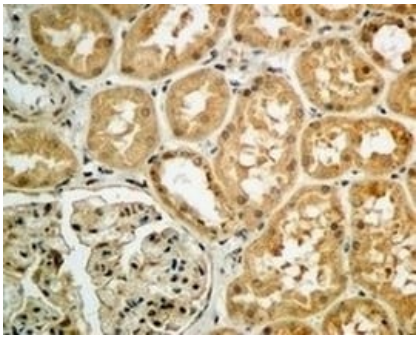
Predicted band size: 97 kDa

Blocking and diluting buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

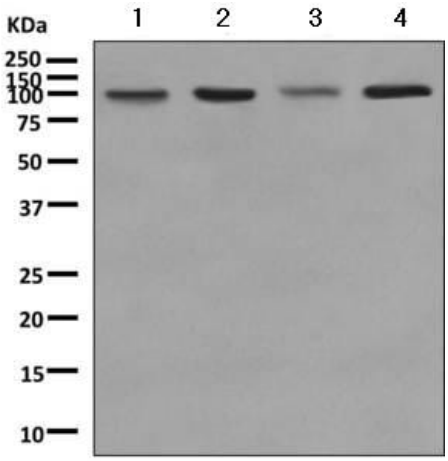
Immunocytochemistry/ Immunofluorescence analysis of MCF-7 (Human breast adenocarcinoma epithelial cell) cells labeling Argonaute-2 with Purified ab156870 at 1:200 dilution (9.5µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling Ago2 / eIF2C2 with unpurified ab156870 at 1/50 dilution.

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Western blot - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

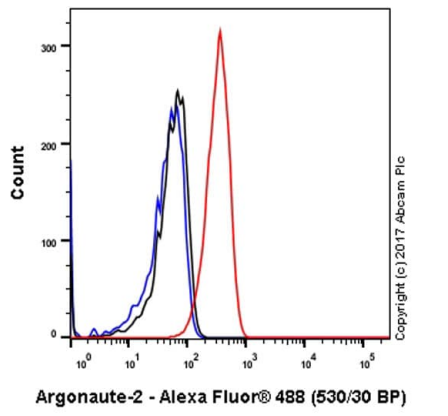
All lanes : Anti-Argonaute-2 antibody [EPR10410] (ab156870) at 1/1000 dilution (unpurified)

Lane 1 : HeLa cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : K562 cell lysate

Lysates/proteins at 10 µg per lane.

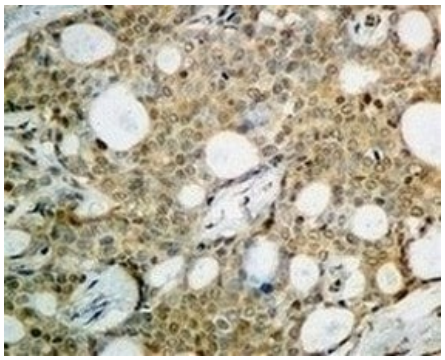
Secondary
All lanes : Goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 97 kDa



Flow Cytometry (Intracellular) - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

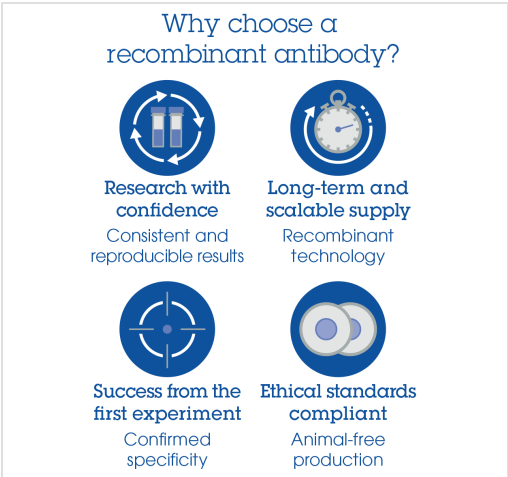
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Argonaute-2 (red) with unpurifiedab156870 at a 1/200 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (**ab172730**). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Argonaute-2 antibody [EPR10410] (ab156870)

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling Ago2 / eIF2C2 with unpurified ab156870 at 1/50 dilution.

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Anti-Argonaute-2 antibody [EPR10410] (ab156870)

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