diagenode
A Hologic Company

TECHNICAL DATASHEET

AGO (Argonautes) antibody

Cat. No. C15200167

Type: Monoclonal
Isotype: IgG1
Source: Mouse
Lot: 004
Size: 50-100 µg
Concentration: 2.4 µg/µl

Specificity: Human, mouse, other (wide range): positive.
Purity: Protein A purified monoclonal antibody.
Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Storage buffer: PBS containing 0.05% azide.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Purified monoclonal antibody prepared by immunizing mice with recombinant human Ago2 protein (GST fusion protein containing amino acids 47–879 of Ago2). The antibody also recognizes other Ago family members although with the highest affinity for Ago2.

Applications

Applications	Suggested dilution	References
IP (RIP)	8 µg per IP	Fig 1
HITS-CLIP	-	Fig 2
Western Blotting	1:500	Fig 3
Immunofluorescence	1:500	Fig 4
IHC	1:500	

Target description

Members of the Argonaute (Ago) protein family are central to RISC function. Argonaute proteins bind to mature microRNA (miRNAs) and short interfering RNAs (siRNAs) and form the core components of effector complexes that mediate miRNA and siRNA function. Argonautes are needed for miRNA-induced silencing and contain two conserved RNA binding domains: a PAZ domain that can bind the single stranded 3' end of the mature miRNA and a PIWI domain that structurally resembles ribonuclease-H and functions to interact with the 5' end of the guide strand.

Some argonautes, for example human Ago2, cleave target transcripts directly; argonautes may also recruit additional proteins to achieve translational repression. The human genome encodes eight Argonaute proteins divided by sequence similarities into two families: AGO (with four members present in all mammalian cells and called E1F2C/hAgo in humans), and PIWI (found in the germ line and hematopoietic stem cells).

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Results

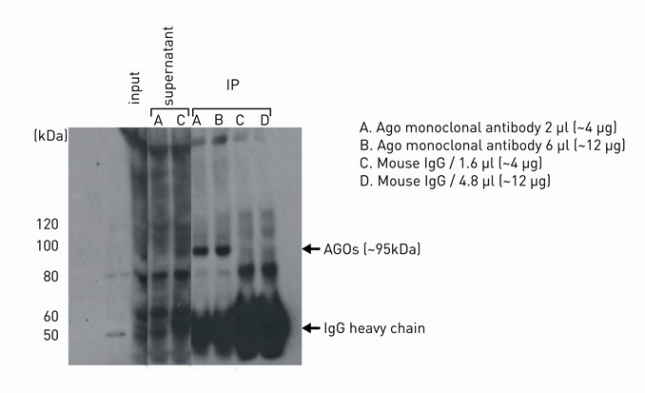

Figure 1. Immunoprecipitation of endogenous Argonaute proteins using the Diagenode Ago monoclonal antibody

Hela cells (10 cm dish per IP) were subjected to UV crosslinking, lysed in 70 µl of 1xPXL buffer (1xPBS, 0.1% SDS, 0.5% Na-deoxycholate, 0.5% NP-40) by incubating on ice for 10 min, then treated with DNase I. Dynabeads Protein A (40 µl each) were first incubated with 12 µg anti-mouse IgG, used as a bridge antibody, briefly washed, and then incubated with Diagenode Ago monoclonal antibody (Cat. No. C15200167) (4 or 12 µg), or non-immune mouse IgG control (4 or 12 µg). The cell lysate and Dynabeads were mixed and incubated at 4°C for overnight. The beads were washed twice each at 4°C for 5 min using 500 µl of (i) 1 x PXL buffer, (ii) 5 x PXL (5xPBS, 0.1% SDS, 0.5% Na-deoxycholate, 0.5% NP-40), and (iii) 1 x PNK (50 mM Tris pH7.4, 10 mM MgCl2, 0.5% NP-40). Ten µl of input (10% of starting material), 10 µl of supernatants after immunoprecipitation (10%), and all of immunoprecipitants (~90%) were loaded on SDS-PAGE gel and subjected to western blot analysis using the Diagenode Ago monoclonal antibody (Cat. No. C15200167) and the ECL plus reagent.

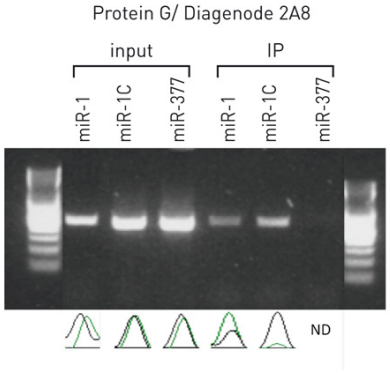

Figure 2. Allelic imbalance Ago-RIP assay with the Diagenode Argonautes monoclonal antibody

The goal of this experiment is to show the preferential coimmunoprecipitation of the miRNA targeted alleles using Diagenode Ago monoclonal antibody (Cat No. C15200167). Briefly, Hela cells were transfected with:

pRL-TK-4A + pRL-TK-4G + pcDNA-miR-1 (miR-1 is supposed to target "A" allele)

pRL-TK-4A + pRL-TK-4G + pcDNA-miR-1C (miR-1C is supposed to target "G" allele)

pRL-TK-4A + pRL-TK-4G + pcDNA-miR-377 (miR-377 is supposed to target neither allele)

Cell lysate was immunoprecipitated with Protein G agarose beads incubated with Diagenode Ago monoclonal antibody. RNA was isolated from the complexes from the input and IP samples, reverse transcribed using random hexamers, and subjected to RT-PCR to confirm enrichment of miRNA targeted allele in IP sample compared to input sample. The PCR products were directly sequenced. The electropherograms at the polymorphic site are shown in the second panel.

"The predicted miRNA target alleles were efficiently co-immunoprecipitated by the Diagenode Ago monoclonal antibody (Cat. No. C15200167).

More details can be found in Takeda H, Charlier C, Farnir F, Georges M., RNA, 2010,1854-63, Epub 2010, Aug 2.

Results were kindly provided by Haruko Takeda (Unit of Animal Genomics, GIGA Research Center, Faculty of Veterinary Medicine, University of Liège, 4000-Liège, Belgium).

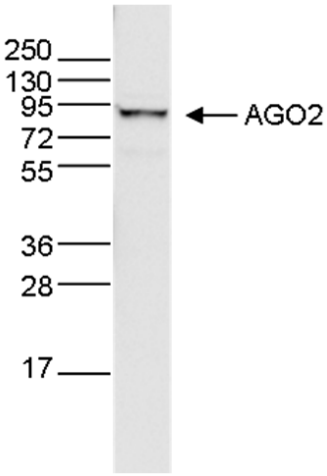

Figure 3. Western blot analysis using the Diagenode Ago monoclonal antibody

Whole cell extracts (40 µg) from HeLa cells were analysed by Western blot using the Diagenode Ago monoclonal antibody (cat. No. C15200167) diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right; the marker (in kDa) is shown on the left.

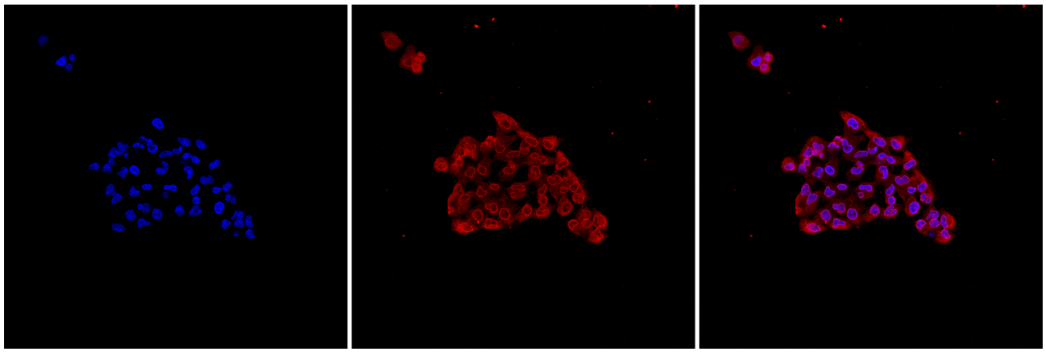

Figure 4. Immunofluorescence using the Diagenode Ago monoclonal antibody

HeLa cells were stained with the Diagenode antibody against Ago (cat. No. C15200167) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labelled with the Ago antibody (middle) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The left panel shows staining of the nuclei with DAPI. A merge of both stainings is shown on the right.