

Datasheet for 200-301-V04**AGO Antibody****Overview**

Description:	Anti-AGO (Argonautes) (MOUSE) Monoclonal Antibody - 200-301-V04
Item No.:	200-301-V04
Size:	50 µg
Applications:	IF, IP, WB
Reactivity:	Human, Mouse, Broad
Host Species:	Mouse

Product Details

Background:	Members of the Argonaute (Ago) protein family are internal 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). Anti-Ago Antibody is ideal for research in Alternative Splicing, Gene Expression and Gene Silencing.
Synonyms:	Protein argonaute-2, Argonaute2, hAgo2, Argonaute RISC catalytic component 2, Eukaryotic translation initiation factor 2C 2, eIF-2C2, eIF2C 2, PAZ Piwi domain protein, PPD, Protein slicer
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	2A8
Format:	IgG1

Target Details

Gene Name:	AGO2
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Reactivity:	Human, Mouse, Broad
Immunogen Type:	Conjugated Peptide
Immunogen:	Anti-Ago Antibody was produced in mice by repeated immunization with human Ago2 protein (fusion protein containing amino acids 47-859 of human Ago2).
Purity/Specificity:	Anti-Ago Antibody was purified by Protein A chromatography. This 2A8 monoclonal antibody recognizes all 4 human and mouse Ago proteins while having higher affinity for Ago2. Cross-reactivity with Ago2 from other sources has not been determined.
Relevant Links:	• UniProtKB - Q9UKV8 • GenelD - 27161 • NCBI - NP_001158095

Application Details

Tested Applications:	IF, IP, WB
Application Note:	Anti-Ago Antibody is tested for HITS-CLIP (High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation), Immunofluorescence, Immunoprecipitation and Western Blots. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 97.2 kDa in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
IF:	1:500
IHC:	1:500
IP:	8 µg
WB:	1:300 - 1:500

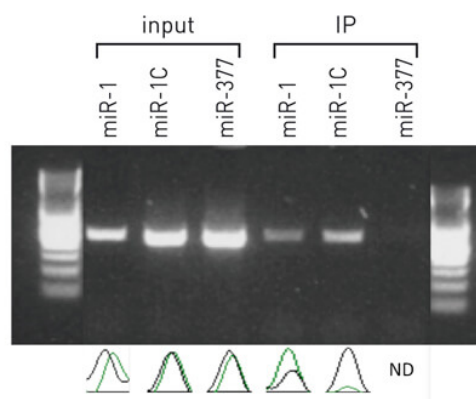
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	2.39 mg/ml by UV absorbance at 280 nm
Buffer:	0.01 M Sodium Phosphate, 0.25 M Sodium Chloride, pH 7.2
Preservative:	0.05% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images

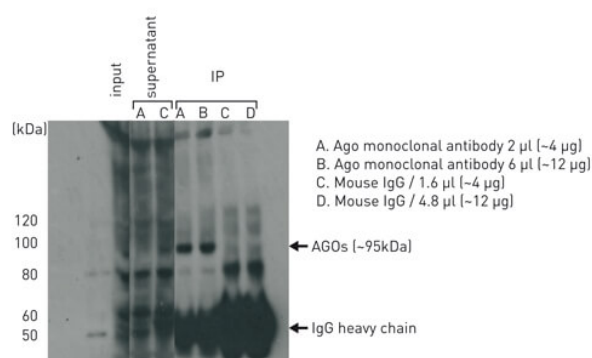


Immunoprecipitation

Co-immunoprecipitation results of Anti-Ago antibody. 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 Anti-Ago antibody. RNA was isolated from the complexes from the input and IP samples, reverse transcribed using random hexamers, and subjected to RTPCR 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 bottom panel. Results provided by Haruko Takeda (University of Liège, Belgium).

Immunoprecipitation

Immunoprecipitation results of Mouse anti-Ago antibody. Load: 10 µl of input (10% of starting material), 10 µl of supernatants after IP (10%), and each IP (~90%). Lane A: 4µg anti-Ago monoclonal antibody. Lane B: 12µg anti-Ago monoclonal antibody. Lane C: 4 µg mouse IgG. Lane D: 12 µg mouse IgG. Primary antibody: Ago antibody at 1:1000 for overnight at 4°C. Secondary antibody: anti-mouse HRP secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO/TBS-Tween overnight at 4°C.



Disclaimer

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