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contact: support@agrisera.com

Agrisera AB | Box 57 | SE-91121 Vännäs | Sweden | +46 (0)935 33 000 | www.agrisera.com

Product no AS09 527 AGO1 | Argonaute 1 Product information

Immunogen	KLH-conjugated, N-terminal peptide of Arabidopsis thaliana AGO1 O04379, At1g48410
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 μg
Reconstitution	For reconstitution add 50 μ l of sterile water
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
Additional information	Antibody binds microRNA and tasiRNAs, preference for 21nt miRNAs with 5'U.
	To detect AGO1 in Nicotiana benthamiana, please inquire.
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Recommended for detection of AGO1: extreme low femtogram range chemiluminescent detection reagent

Application information

Recommended dilution	2 μg (ChIP), 1: 200 (IF), (IL), small-RNA-IP-Seq, 1 : 5000-1 : 10 000 (WB)
Expected apparent MW	116.4 130 kDa
Confirmed reactivity	Arabidopsis thaliana, Hyacinthus orientalis, Nicotiana benthamiana
Predicted reactivity	Brassica pekinensis, Capsella rubella, Cucumis sativus, Glycine max, Malus domestica, Pisum sativum, Ricinus communis, Solanum lycopersicum, Solanum tuberosum, Vitis vinifera Species of your interest not listed? <u>Contact us</u>
Not reactive in	Chlamydomonas reinhardtii, Medicago sativa, Salvia sp., Spinacia oleracea, Oryza sativa, Phaseolus vulgaris, Triticum aestivum, Zea mays
Additional information	AGO expression may be tissue specific and using floral tissue is recommended where most of the AGOs are expressed the highest. Use of proteasome inhibitors as MG132 can help to stabilize AGO proteins during extraction procedure. Buffer for extraction of AGO proteins: <u>Paudel</u> et al. 2018.
	The AGO1 antibody is extremely specific to AGO1 and does not cross-react with other antibodies. The evidence is 1) the peptide to which it was raised is at the very N-terminus of the protein and is not present in other AGOs 2) aAGO1 does not cross react with the AGOs which are overexpressed (AGO2, AGO3, AGO4, AGO5, AGO6, AGO9) using a western blot.
	TCA acetone precipitation method
Selected references	Bradamante et al. (2023). Two ARG ONAUTE proteins loaded with transposon-derived small RNAs are associated with the reproductive cell lineage in Arabidopsis. Plant Cell. 2023 Dec 7:koad295.doi: 10.1093/plcell/koad295. Meng et al. (2022) The novel activity of Argonautes in intron splicing: A transcriptome-wide survey in plants. J Plant Physiol. 2022 Jan 31;270:153632. doi: 10.1016/j.jplph.2022.153632. Epub ahead of print. PMID: 35114616. Cabezas-Fuster et al. (2022). Missplicing suppressor alleles of Arabidopsis PRE-MRNA PROCESSING FACTOR 8 increase splicing fidelity by reducing the use of novel splice sites. Nucleic Acids Res. 2022 Jun 10;50(10):5513-5527. doi: 10.1093/nar/gkac338. PMID: 35639749; PMCID: PMC9177961. Delenko et al. (2022) MicroRNA biogenesis and activity in plant cell dedifferentiation stimulated by cell wall removal. BMC Plant Biol. 2022 Jan 3;22(1):9. doi: 10.1186/s12870-021-03323-9. PMID: 34979922; PMCID: PMC8722089. (immunofluorescence) Oliver & Martinez. (2021) Accumulation dynamics of ARGONAUTE proteins during meiosis in Arabidopsis. Plant Reprod. 2021 Nov 23. doi: 10.1007/s00497-021-00434-z. Epub ahead of print. PMID: 34812935. Dalmadi et al. (2021) Controlled RISC loading efficiency of miR168 defined by miRNA duplex structure adjusts ARGONAUTE1 homeostasis. Nucleic Acids Res. 2021 Dec 16;49(22):12912-12928. doi: 10.1093/nar/gkab1138. PMID: 34850097; PMCID: PMC8682782.

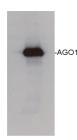


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Application example



80 µg of *Arabidopsis thaliana* soluble total cell extract (extracted in 20 mMTris pH 7.5, 5mM MgCl₂, 2.5mM DTT, 300 mM NaCl, 0.1% NP-40, 1% proteasome inhibitor MG132) was separated on 6% SDS-PAGE and blotted 1h to nitrocellulose. Filters were blocked 1h with 5% low-fat milk powder in TBS-TT (0.25% TWEEN20; 0.1% Triton-X) and probed with anti-AGO1 antibody (1:10 000, 1h) and secondary anti-rabbit (1:10000, 1 h) antibody (HRP conjugated) in TBS-TT containing 5% low fat milk powder. Antibody incubations were followed by washings in TBS-TT. All steps were performed at RT with agitation. Blots were developed for 5 min with ECL-PLUS detection reagent according to the manufacturer's instructions. Exposure time was 5 seconds.

Roche's protease inhibitor cocktail (no EDTA) can also be applied in extraction buffer.

Courtesy Dr. Ericka Havecker, University of Cambridge, UK