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Product no AS09 617

Anti-AGO4 | Argonaute 4

Product information

Immunogen	KLH-conjugated synthetic peptide chosen from *Arabidopsis thaliana* AGO4 sequence UniProt: Q9ZVD5 , TAIR: At2g27040
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity purified serum in PBS pH 7.4.
Format	Lyophilized
Quantity	50 µg
Reconstitution	For reconstitution add 50 µl of sterile water
Storage	Store lyophilized/reconstituted at -20°C; Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur adhering to the cap or sides of the tube.

Application information

Recommended dilution	1 : 100 (ICC), 5 µg of antibody per 1 gram of a fresh tissue (IP),1 : 2000-1 : 5000 (WB)
Expected | apparent MW	103 kDa
Confirmed reactivity	*Arabidopsis thaliana, Hyacinthus orientalis*
Predicted reactivity	*Arabidopsis thaliana*
Not reactive in	*Brassica oleracea, Fragaria x ananassa, Hordeum vulgare, Solanum lycopersicum, Vigna angularis, 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. Use a 6% gel for protein separation, which is run longer to avoid a cross-reactivity at ca. 40 kDa. Binds endogenous siRNAs, preference for 24nt siRNAs with 5' A. Note that the AGO4 antibody reacts with the NEB prestained protein marker.
Selected references	Miloro et al. (2024). Barley AGO4 proteins show overlapping functionality with distinct small RNA-binding properties in heterologous complementation. Plant Cell Rep. 2024 Mar 13;43(4):96. doi: 10.1007/s00299-024-03177-z. Clavel et al. (2021) Atypical molecular features of RNA silencing against the phloem-restricted polerovirus TuYV. Nucleic Acids Res. 2021 Nov 8;49(19):11274-11293. doi: 10.1093/nar/gkab802. PMID: 34614168; PMCID: PMC8565345. 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. Niedojadlo et al. (2020). Dynamic distribution of ARGONAUTE1 (AGO1) and ARGONAUTE4 (AGO4) in Hyacinthus orientalis L. pollen grains and pollen tubes growing in vitro. Protoplasma. 2020 Jan 8. doi: 10.1007/s00709-019-01463-2. Sprunck et al. (2019). Elucidating small RNA pathways in Arabidopsis thaliana egg cells. http://dx.doi.org/10.1101/525956 Yang et al. (2017). The developmental regulator PKL is required to maintain correct DNA methylation patterns at RNA-directed DNA methylation loci. Genome Biol. 2017 May 31;18(1):103. doi: 10.1186/s13059-017-1226-y.

Application example

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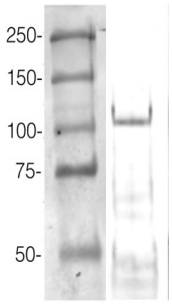

360 µg/well of *Arabidopsis thaliana* protein extracted by TCA-acetone precipitation from floral tissue and saturated in 8M urea were separated on 15% SDS-PAGE and blotted for 1hour to 0.2 µm nitrocellulose at 100V using wet transfer system. Blots were blocked with 0.5% cold fish gelatin for 1hr at room temp with agitation. Blot was incubated in the primary antibody at a dilution of 1:2500 for an hour at RT with agitation. The blots were washed with 3X 15min TBS-TT at RT with agitation. Blots as incubated in the secondary antibody (goat anti-rabbit DyLight® 800 conjugated, AS12 2460, Agrisera) 1:5000 dilution for 30 min. at RT with agitation and washed 1X with TBSTT for 15 min, 1X with TBST for 15min before scanning with the ODyssey IRD scanner.

Courtesy of Dr. Betty Chung and Pawel Baster, University of Cambridge, United Kingdom