

product **AS09 527**
AGO1 | argonaute 1

product information

background	AGO1 belongs to a group of argonaute proteins which are catalytic component of the RNA-incudes silencing complex (RISC). This protein complex is responsible for the gene silencing (RNAi).
immunogen	N-terminal peptide of <i>Arabidopsis thaliana</i> AGO1 O04379
antibody format	rabbit polyclonal affinity purified serum lyophilized
quantity	100 µg for reconstitution add 100 µl of sterile water.
storage	store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.
tested applications	western blot (WB), immunoprecipitation (IP)
additional information	antibody binds microRNA and tasiRNAs, preference for 21nt miRNAs with 5'U

application information

recommended dilution	1: 5000 - 1: 10 000 (ECL Plus), 5 µg of antibody per gram of tissue (IP)
expected apparent MW	116.4 177 kDa
confirmed reactivity	<i>Arabidopsis thaliana</i>
predicted reactivity	<i>Pisum sativum</i> , <i>Ricinus communis</i> , <i>Vitis vinifera</i>
not reactive in	no confirmed exceptions from predicted reactivity known in the moment
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.
selected references	Baumberger et al. (2007). The poliovirus silencing suppressor PO targets ARGONAUTE proteins fo degradation. Current Biology 17: 1609-1614.

application example

80 µg of *Arabidopsis thaliana* soluble total cell extract (extracted in 20mM Tris pH7.5, 5mM MgCl₂, 2.5mM DTT, 300mM NaCl, 0.1% NP-40, 1% proteaseinhibitor) 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, Santa Cruz(sc-2054)) 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 (GE Healthcare). Exposure time was 5 seconds.

Courtesy Dr. Ericka Havecker, University of Cambridge

