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Product no **AS10 710**

H3 | Histone H3 (rabbit antibody) (nuclear marker)

Product information

Immunogen	KLH-conjugated synthetic peptide derived from known H3 sequences, including <i>Arabidopsis thaliana</i> H3.3 P59169 (At4g40030 , At4g40040 , At5g10980), H3.2 P59226 (At1g09200 , At3g27360 , At5g10390 , At5g10400 , At5g65360), H3-like 2 Q9FXI7 (At1g19890)
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 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.

Additional information Cellular **compartment marker** of nucleoplasm, loading control antibody for *Chlamydomonas reinhardtii*

Application information

Recommended dilution	1 : 100-1 : 500 (ICC), 2 µl of antibody/500 µl solution (Chlp-qPCR), 1: 500 (IF), 1 : 5000 (WB)
Expected apparent MW	15 17 kDa
Confirmed reactivity	<i>Arabidopsis thaliana</i> , <i>Brassica oleracea</i> , <i>Capsicum annuum</i> , <i>Cicer arietinum</i> L., <i>Chlamydomonas acidophila</i> , <i>Chlamydomonas reinhardtii</i> , <i>Cucumis sativus</i> L cv <i>Suyo</i> , <i>Hordeum vulgare</i> , human, <i>Nicotiana benthamiana</i> , <i>Phalaenopsis equestris</i> , <i>Physcomitrella patens</i> , <i>Salicornia europaea</i> , <i>Solanum lycopersicum</i> , <i>Solanum sogardinum</i> , <i>Solanum tuberosum</i> , <i>Vicia faba</i> , <i>Zea mays</i>
Predicted reactivity	<i>Brachypodium distachyon</i> , <i>Brassica napus</i> , <i>Emiliana huxleyi</i> , <i>Nannochloropsis gaditana</i> , <i>Nicotiana tabacum</i> , <i>Malus domestica</i> , <i>Medicago sativa</i> , <i>Triticum aestivum</i> , <i>Pinus pinaster</i> , <i>Pisum sativum</i> , <i>Oryza sativa</i> , <i>Ostreococcus sp.</i> , <i>Vitis vinifera</i> , <i>Volvox sp.</i>
	Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known.
Additional information	Specific fluorescence in ICC has been observed for interphase nuclei as well as around centromer region (where Ser10 of histone H3 is phosphorylated) in mitotic chromosomes. For high resolution images, please visit the specific product page at www.agrisera.com
Selected references	Chen et al. (2019) . Phalaenopsis LEAFY COTYLEDON1-Induced Somatic Embryonic Structures Are Morphologically Distinct From Protocorm-Like Bodies. <i>Front Plant Sci.</i> 2019 Nov 29;10:1594. doi: 10.3389/fpls.2019.01594. PMID: 31850050; PMCID: PMC6896055. Perlaza et al. (2019) . The Mars1 kinase confers photoprotection through signaling in the chloroplast unfolded protein response. <i>Elife.</i> 2019 Oct 15;8. pii: e49577. doi: 10.7554/eLife.49577. (immunofluorescence) Dalmadi et al. (2019) . AGO-unbound cytosolic pool of mature miRNAs in plant cells reveals a novel regulatory step at AGO1 loading. <i>Nucleic Acids Res.</i> 2019 Aug 8. pii: gkz690. doi: 10.1093/nar/gkz690. Barua et al. (2019) . Dehydration-responsive nuclear proteome landscape of chickpea (<i>Cicer arietinum</i> L.) reveals phosphorylation-mediated regulation of stress response. <i>Plant Cell Environ.</i> 2019 Jan;42(1):230-244. doi: 10.1111/pce.13334. Du et al (2019) . Proteomic identification of lipid-bodies-associated proteins in maize seeds. <i>Acta Physiologiae Plantarum</i> , May 2019, 41:70

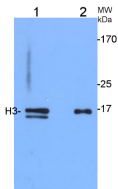
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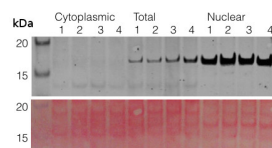
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Application example



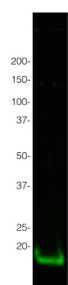
1.2 μg of *Arabidopsis thaliana* chromatin-enriched fraction (1) and 3.75 μg of total protein from 4-week-old *Arabidopsis thaliana* leaves (2), and were separated on 12% SDS-PAGE and blotted 50 mins to Immobilon-P (Millipore, semi-dry) PVDF membrane. Blots were blocked immediately following transfer in MTBS-T (5% milk) for 30 mins at room temperature with agitation. Blots were incubated in the primary antibody at a dilution of 1:5000 for 1h at room temperature with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 3 times for 3 min in TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti- IgG horse radish peroxidase conjugated, from Agrisera, [AS09 602](#)) diluted to 1:20 000 for 30 mins at room temperature with agitation. The blots were washed as above and developed for 5 min with ECL detection reagent according to the manufacturers instructions. Exposure time was 30 seconds. Double band in chromatine-enriched fraction (1) has been outcompeted in peptide neutralization assay by peptide used to elicit H3 antibodies. Chromatin izolation was carried out as described ([Zilberman et al. 2008](#)) with minor modifications.

Courtesy of Weronika Sura and Dr. Piotr A. Ziolkowski, Department of Biotechnology, Adam Mickiewicz University, Poznan, Poland



30 μg of 5 μl of *Chlamydomonas reinhardtii* protein saturated in 8M urea were separated on 15% SDS-PAGE and blotted for 1 hour 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 (anti-H3) 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 (DayLight 800) 1:5000 dilution for 30min at RT with agitation and washed 1X with TBSTT for 15min, 1X with TBST for 15min before scanning with the ODyssey IRD scanner.

Courtesy of Dr. Betty Chung, University of Cambridge, United Kingdom



5 μl of 15 $\mu\text{g}/\mu\text{l}$ *Solanum lycopersicum* protein saturated in 8M urea were separated on 15% SDS-PAGE and blotted for 1 hour 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 (anti-H3) 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, fluorescent antibody ([AS12 2460](#), Agrisera) 1:5000 dilution for 30min at RT with agitation and washed 1X with TBSTT for 15min, 1X with TBST for 15min before scanning with the ODyssey IRD scanner.

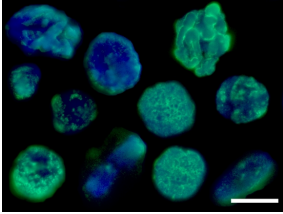
Courtesy of Dr. Betty Chung and Dr Zhengming Wang, University of Cambridge, United Kingdom

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Immunocytochemical assays were performed according to the method described earlier (Rybaczek and Maszewski 2006). Excised apical parts of *Vicia faba* roots (1.5 mm long) were fixed for 45 min (18 °C) in PBS-buffered 3.7% paraformaldehyde, washed several times with PBS and placed in a citric acid-buffered digestion solution (pH 5.0; 37 °C for 45 min) containing 2.5% pectinase (Fluka), 2.5% cellulase (Onozuka R-10; Serva) and 2.5% pectoliase (ICN). After removing the digestion solution, root tips were washed 3 times in PBS, rinsed with distilled water and squashed onto Super Frost Plus glass slides (Menzel-Gläser). Air-dried slides were pretreated with PBS-buffered 5% BSA at 20 °C for 50 min and incubated overnight in a humidified atmosphere (4 °C) with rabbit antibody raised against H3 histone (Agrisera), dissolved in PBS containing 1% BSA (at a dilution of 1:50). Following incubation, slides were washed 3 times with PBS and incubated for 1 h (18 °C) with Agrisera secondary goat anti-rabbit IgG DyLight®488 antibody ([AS09_633](#), 1:1000). Nuclear DNA was stained with 4',6-diamidino-2-phenyl-indole (DAPI, 0.4 µg/ml; Sigma-Aldrich). Following washing with PBS, slides were air dried and embedded in Vectashield Mounting Media for Fluorescence (Vector Laboratories). Observations were made using Optiphot-2 fluorescence microscope (Nikon) equipped with B-2A filter (blue light; 495 nm) for DyLight-conjugated antibodies and UV-2A filter (UV light; 365 nm) for DAPI. All images were recorded at exactly the same time of integration using DXM 1200 CCD camera.

Courtesy Dr. Dorota Rybaczek, Łódź University, Poland