

product **AS10 710**  
**H3 | histone H3**

### product information

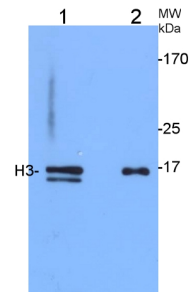
<b>background</b>	<b>Histone 3 (H3)</b> located in nuclei, incorporated into chromatin. Present in nucleosome together with H2A, H2B and H4.
<b>immunogen</b>	<u>KLH</u> -conjugated synthetic peptide derived from known H3 sequences, including <i>Arabidopsis thaliana</i> H3.3 <a href="#">P59169</a> , H3.2 <a href="#">P59226</a> , H3-like 2 <a href="#">Q9FX17</a>
<b>antibody format</b>	rabbit polyclonal serum lyophilized
<b>quantity</b>	100 µl for reconstitution add 100 µl, of sterile water.
<b>storage</b>	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.
<b>tested applications</b>	western blot (WB), immunocytochemistry (ICC)
<b>additional information</b>	cellular <b>[compartment marker]</b> of nucleoplasm

### application information

<b>recommended dilution</b>	1 : 5000 with standard ECL (WB), 1: 100 - 1: 500 (ICC)
<b>expected   apparent MW</b>	15   17 kDa
<b>confirmed reactivity</b>	<i>Arabidopsis thaliana</i> , <i>Chlamydomonas reinhardtii</i> , <i>Salicornia europaea</i> , <i>Solanum lycopersicum</i> , <i>Vicia faba</i> , <i>Zea mays</i>
<b>predicted reactivity</b>	dicots including: <i>Vitis vinifera</i> , monocots: <i>Hordeum vulgare</i> , <i>Oryza sativa</i> , <i>Zea mays</i> , trees: <i>Pinus pinaster</i> , moss: <i>Physcomitrella patens</i> , algae: <i>Volvox sp.</i>
<b>not reactive in</b>	no confirmed exceptions from predicted reactivity known in the moment
<b>additional information</b>	Protocol for isolation of cytosolic and nuclear fractions can be found <a href="#">here</a> . 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.
<b>selected references</b>	<a href="#">Linde et al. (2012)</a> . A Maize Cystatin Suppresses Host Immunity by Inhibiting Apoplastic Cysteine Proteases. <i>Plant Cell</i> , March 27, ahead of print. <a href="#">Fang et al. (2011)</a> . Coordination of Carbon Fixation and Nitrogen Metabolism in <i>Salicornia europaea</i> under Salinity: Comparative Proteomic Analysis on Chloroplast Proteins. <i>Proteomics</i> Sep 9.

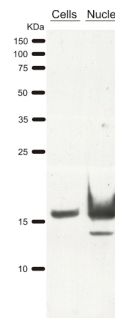
## application example

**1.2 µg** of *Arabidopsis thaliana* chromatin-enriched fraction (1) and 3.75 µg of total protein from 4-weeks-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 (Roche) 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.

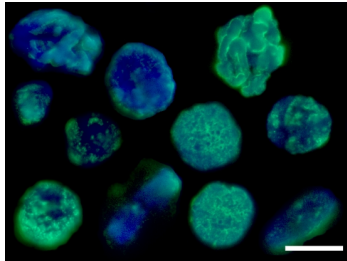


Courtesy of Weronika Sura and Dr. Piotr A. Ziolkowski (Department of Biotechnology, Adam Mickiewicz University, Poznan, Poland)

**30 µg** of *Chlamydomonas reinhardtii* total ("Cells") or nuclei-enriched ("Nuclei") proteins were separated on a 13% SDS-PAGE gel and blotted 90 minutes to AmershamTM HybondTM-ECL (pore size 0.2 µm). Membranes were blocked in TBST 5% milk for one hour at room temperature and incubated at 4°C overnight in TBST 5% milk with the Agrisera primary H3 antibody at a dilution of 1:5000. After three washes in TBST, blots were incubated one hour at room temperature with Agrisera HRP-conjugated Goat anti-rabbit IgG (H&L) diluted 1:50000 in TBST 5% milk. Signal was visualized with standard ECL on Amersham HyperfilmTM ECL after 1 minute exposure. Molecular weight is determined by the Broad Range Protein Molecular Weight Marker (Promega).



Courtesy of Dr. Leonardo Magneschi, Hippler's Lab, Institut für Biologie und Biotechnologie der Pflanzen (IBBP), Westfälische Wilhelms-Universität Münster, Germany



**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, Lodz University, Poland