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Product no AS10 710A

H3 | Histone H3 (antigen affinity purified)

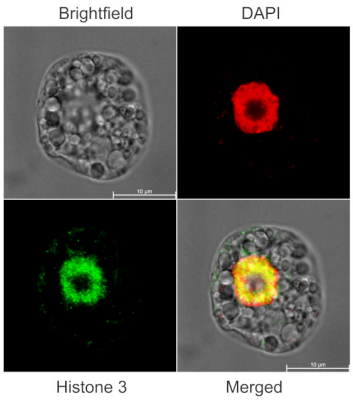
Product information

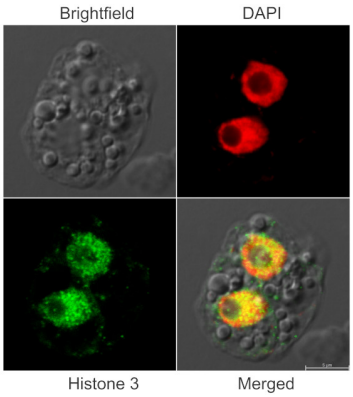
Immunogen	KLH-conjugated synthetic peptide derived from known H3 sequences, inluding *Arabidopsis thaliana* H3.3 P59169 (At4g40030,At4g40040,At5g10980), H3.2 P59226(At1g09200,At3g27360,At5g10390,At5g10400,At5g65360), H3-like 2 Q9FXI7 (At1g19890)
Host	Rabbit
Clonality	Polyclonal
Purity	Immunogen affinity 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; 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	Cellular [compartment marker] of nucleoplasm, loading control antibody for *Chlamydomonas reinhardtii*

Application information

Recommended dilution	2,5 µg/100 µg of chromatin (ChIp-qPCR), 1: 400 (IF), 1 : 5000 (WB)
Expected | apparent MW	15 | 17 kDa
Confirmed reactivity	*Arabidopsis thaliana, Oryza sativa*
Predicted reactivity	*Brassica oleracea, Capsicum annuum, Chlamydomonas acidophila, Chlamydomonas reinhardtii, Physcomitrium patens, Salicornia europaea, Solanum lycopersicum, Solanum sogarandinum, Solanum tuberosum, Vicia faba, Zea mays Brachypodium distachyon, Brassica napus, Hordeum vulgare, Nicotiana tabacum, Malus domestica, Medicago sativa, Nannochloropsis gaditana, Triticum aestivum, Pinus pinaster, Pisum sativum, Zea mays, Vitis vinifera, Volvox sp.* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	Protocol for isolation of cytosolic and nuclear fractions can be found here. Specific protol for ChIP can be found here: Saleh et al. (2008).

Application example

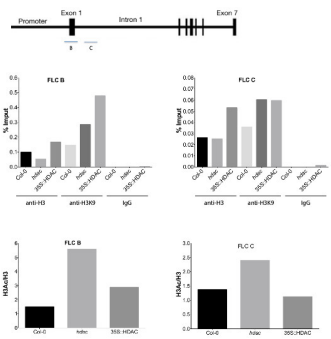




Immunofluorescent localization of Histone 3 on suspension culture of *Arabidopsis thaliana* (upper image) *or Oryza sativa* (bottom image), using anti-histone 3 antibodies (AS10 710A) and anti-rabbit IgG DyLight®488 conjugated secondary antibodies (AS10 1165). DAPI staining of nuclei is pseudocolored red.

Material: Suspension cultures of *Arabidopsis thaliana,* ecotype Landsberg erecta cv.MM1 or *Oryza sativa* ssp.japonica cv. 'Unggi 9'
Fixation: Packed cell volume to fixer ratio: 250 µl : 5ml
Fixer composition and buffer: 4% (w/v) paraformaldehyde (freshly prepared as 8% stock and 0.2 µm filtered) in Phosphate Buffered Saline (PBS), pH 7.4 (2x stock, 0.2 µm filtered)
Container and method: in 6 cm Petri dish, gentle shaking at room temperature (RT)
Duration: 30 minutes (*Arabidopsis thaliana*) or 60 minutes (*Oryza sativa*). Cells were not shaken during the first 5 mins of fixation to allowed to partially recover from osmotic shock induced by formaldehyde.
Hydrophilization: no
Cell wall digestion: Yes
Packed cell volume to enzyme ratio: 100 µl : 2ml Enzyme composition: 1% (A) 1.2% (R) Cellulase (chromatically purified, powder, Worthington) 1% Pectinase (protease free, liquid, Sigma) Buffer: 0.5% (w/v) MES buffer, pH 5.6
Container and method: in 2 ml microfuge tube by rolling at room temperature (RT)
Duration: 30 minutes (*Arabidopsis thaliana*) or 90 minutes (*Oryza sativa*)
Membrane permeabilization: Triton-X100 (0.5%), 10 min/RT
Antigen retrieval: no
Blocking buffer: Fish gelatin (5% v/v)
Washing buffer: PBS
Primary antibody dilution and incubation time: 1:400, ON/4ºC
Secondary antibody dilution and incubation time and supplier: anti-rabbit IgG DyLight®488 conjugated secondary antibodies (AS10 1165), 1:600, 1hn/RT
Co-staining of the nucleus (DAPI): Yes
Nucleus staining: 100 ng/ml DAPI

Courtesy of Dr. Ferhan Ayaydin, Hungarian Centre of Excellence for Molecular Medicine (HCEMM), Szeged, Hungary.



Chromatin Immunoprecipitation: using anti-plant Histone 3 polyclonal antibodies. Chromatin from *Arabidopsis thaliana* wilde type, deacetylase mutant and over-expressors was cross-linked using formaldehyde. Chromatin was isolated and DNA was sheared along with the bound protein by sonictaion. DNA-protein complex was immunoprecipitated using affinity purified, polyclonal anti-Histone 3 antibodies. Immunoprecipitated DNA was quantified using quantitative PCR and normalized to the input chromatin.
Procedure was according to a protocol described here: Saleh et al. (2008).

Courtesy of Dr. Cristián Holzmann, Catholic University of Chile, Chile