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Product no AS10 1116

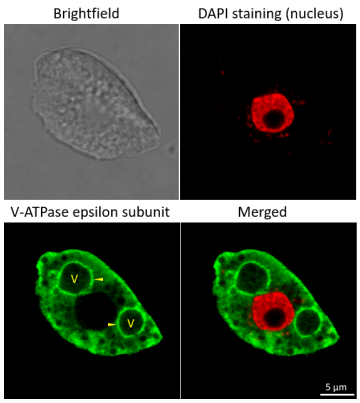
Donkey anti-Goat IgG (H&L), DyLight® 488 conjugated

Product information

Immunogen	Purified Goat IgG, whole molecule
Host	Donkey
Clonality	Polyclonal
Purity	Immunogen affinity purified donkey IgG.
Format	Lyophilized
Quantity	1 mg
Reconstitution	For reconstitution add 1.1 ml of sterile water. Let it stand 30 minutes at room temperature to dissolve. Prepare fresh working dilutions daily
Storage	Store lyophilized material at 2-8°C. Product is stable for 4 weeks at 2-8°C after rehydration. For long time storage after reconstitution, dilute the antibody solution with glycerol to a final concentration of 50% glycerol and store as liquid at -20°C, to prevent loss of enzymatic activity. For example, if you have reconstituted 1 mg of antibody in 1.1 ml of sterile water add 1.1 ml of glycerol. Such solution will not freeze in -20°C, If you are using a 1:5000 dilution prior to diluting with glycerol, then you would need to use a 1:2500 dilution after adding glycerol. Prepare working dilution prior to use and then discard. Be sure to mix well but without foaming.
Additional information	Conjugate is present in 10 mM Sodium Phosphate, 0.15 M Sodium Chloride, pH 7.2, 1 % (w/v) BSA, Protease/IgG free. 0.05 % (w/v) sodium azide is added as preservative. Based on immunoelectrophoresis, this antibody reacts with:, heavy chains on goat IgG, light chains on all goat immunoglobulins No reactivity is observed to, non-immunoglobulin goat serum proteins BSA and milk have to be replaced by other blocking reagents, like doneky serum or commercial formulations which are free from bovine IgG.

Application information

Recommended dilution	1 : 20-1 : 2000 for most applications



Immunofluorescent localization of V-ATPase epsilon subunit of tonoplast H+ATPase in suspension culture of *Oryza sativa* ssp. japonica cv. 'Unggi 9', using goat anti-V-ATPase, epsilon subunit of tonoplast antibodies (AS09 577A) and donkey anti-Goat IgG (H&L), DyLight® 488 conjugated secondary antibodies (AS10 1116, Agrisera). Vacuolar membrane, tonoplast, is highlighted by yellow arrowheads. DAPI staining of nuclei is pseudocolored red.

Method

Material: Suspension cultures of *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) 0.01% (v/v) Triton-X100 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: 40 min
Hydrophilization: No

Cell wall digestion: Yes Packed cell volume to enzyme ratio: 100 µl : 2 ml Enzyme composition: 1% (A) 1.2% (R) Cellulase (chromatically purified, powder, Worthington) 1% (A) 1.2% (R) 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: 60 min
Membrane permeabilization: Triton-X100 (0.35%), 7 min/RT
Antigen retrieval: No
Blocking buffer: Fish gelatin (5% v/v)
Washing buffer: PBS
Primary antibody dilution and incubation time: 1:600, 4ºC/ON
Secondary antibody: donkey anti-Goat IgG (H&L), DyLight® 488 conjugated secondary antibodies (AS10 1116, Agrisera), 1:600, 1h/RT
Co-staining of the nucleus (DAPI): Yes
Cell wall and nucleus staining: 100 ng/ml DAPI

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