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Product no AS16 3224-1ml

Rhamnogalacturonan-I backbone (clone CCRC-M35)

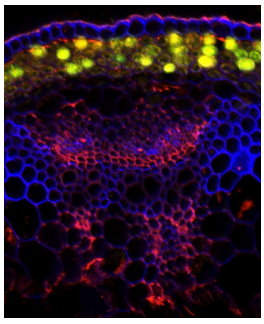
Product information

Immunogen	MeBSA-conjugated *Arabidopsis thaliana* seed mucilage (Rhamnogalacturonan I), non-covalent complex. Epitope structure for carabohydrate antigen: Rha-(1,4)-GalA-(1,2)-Rha-(1,4)-GalA-(1,2)-Rha-(1,4).
Host	Mouse
Clonality	Monoclonal
Subclass/isotype	IgM
Purity	Cell culture supernatant.
Format	Liquid
Quantity	1 ml
Storage	Antibody can be stored up to 1 month at 4°C, and at -80°C for up to 1 year. 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	Exact working dilution needs to be determined by end user

Application information

Recommended dilution	Undiluted or at 1 : 10 (ELISA), (IHC), (IF)
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	Dicots Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known
Additional information	CCRC-M35 binds to the backbone of rhamnogalacturonan I and requires at least two unbranched disaccharide repeats for binding, CCRC-M35 does not bind to branched sections of the backbone and is not sensitive to the identity of the sugar at the non-reducing terminus
Selected references	Pattathil et al. (2012). Immunological approaches to plant cell wall and biomass characterization: Glycome Profiling. Methods Mol Biol. 2012;908:61-72.doi: 0.1007/978-1-61779-956-3_6. Patathil et al. (2010). A comprehensive toolkit of plant cell wall glycan-directed monoclonal antibodies. Plant Physiol. 2010 Jun;153(2):514-25.doi: 10.1104/pp.109.151985.

Application example



Localization of rhamnogalacturonan-I backbone (red) in *Arabidopsis thaliana* hypocotyl, Calcufluor White counterstain (blue) and cell wall autofluorescence (yellow).

The 31 days-old hypocotyls were immersed in 150 µL PME fixation buffer (25 mM PIPES, 1 mM $MgSO_4$, 1 mM EGTA) and then subjected to three consecutive cycles of 5 min-long vacuum infiltration (21°C, 68 kPa). Afterwards they were washed three times in PME (21°C, 68 kPa) prior to storage at 4°C in PME. Hypocotyls were encased in 1 cm^3 blocks of 5% agar at 65°C, and stored at 4°C to set. Transverse 40 µm thick sections were cut from segments using a VT100S vibrating microtome (Leica) and blocked for at least 1 h in 5% bovine serum albumin in TBST. Blocking solution was discarded and sections were incubated at 4°C for 16 h with 5 µl of the anti-Rhamnogalacturonan-I backbone antibody, followed by 2 washes in 100 µL TBST. Sections were then incubated for 1 h at 21°C in the dark in 10 µl of 2 µg/µl Alexa FluorTM 568 donkey anti-mouse IgG (H+L; 1:36). Sections were again washed twice in 40 µL TBST prior to counter-staining with 0.015% Calcofluor White (Sigma-Aldrich). Sections were again washed twice in 100 µL TBST to remove excess counter-stain and unbound secondary antibody. Immunofluorescence of AlexaFluor 568 was excited with a 561 nm laser, and emitted light filtered at 575–600 nm, while Calcufluor White was subsequently scanned on an

independent channel with a 405 nm laser and emission observed at 420–430 nm using laser scanning microscope Zeiss LSM780 point-scan system at 1024 × 1024 pixels (pixel size, 0.6–0.83 µm) with a 10X objective.

Courtesy Dr. Urs Fisher, Umeå Plant Science Centre, Sweden