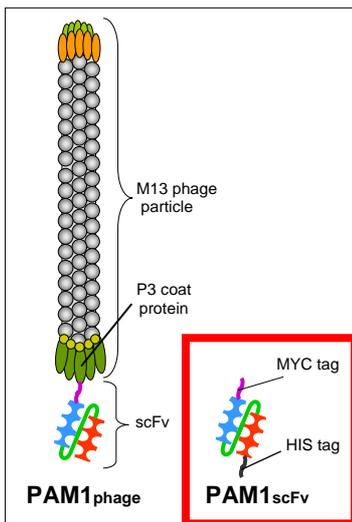


PlantProbes PAM1 antibody data sheet

Monoclonal antibody to HOMOGALACTURONAN Phage display-derived soluble scFv

Introduction: PAM1 is an **anti-homogalacturonan** phage display monoclonal antibody that was isolated from the Synthetic scFv Library #1 (1). PAM1 was first isolated as a M13 filamentous bacteriophage with a single chain antibody fragment (scFv) expressed at its surface (2). This version of the antibody is known as PAM1_{phage}. Only the scFv is required for binding and we have now produced PAM1 as a recombinant, soluble scFv and this version of the antibody is known as PAM1_{scFv} (3). PAM1_{phage} and PAM1_{scFv} have identical specificities. PAM1_{scFv} is a polypeptide of approximately 30 KD and consists of heavy and light chain antibody fragments joined by a glycine-rich linker. PAM1_{scFv} has a N-terminal polyhistidine (HIS) tag and a C-terminal MYC tag.



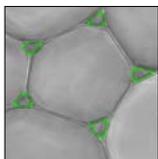
PlantProbes PAM1 is PAM1_{scFv}.

Specificity: PAM1 binds to long stretches of unesterified homogalacturonan and in the region of 30 contiguous nonmethylated galacturonic acid residues are required for binding (2-4).

Storage: PAM1_{scFv} is provided in 50 µg units of freeze-dried protein. To reconstitute the antibody add 500 µl of de-ionised water to each tube. The reconstituted antibody should then be aliquoted and stored at -20°C until needed.

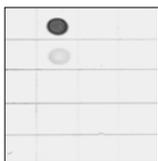
Using PAM1_{scFv}: PAM1_{scFv} can be used in the same range of applications as hybridoma monoclonal antibodies. PAM1_{scFv} binding is detected using the HIS or MYC tags. Shown below are possible outline protocols for the use of PAM1_{scFv} with anti-HIS tag reagents for immunocytochemistry and immuno-dot-assays. Concentrations of PAM1_{scFv} and dilution factors of secondary antibodies are given as a guide but optimum concentrations are likely to vary depending on the samples tested and should therefore be determined empirically for each case.

For blocking and all antibody dilutions we use PBS containing 5% (w/v) fat-free dried milk powder. All incubation steps are between 1 and 2 hours and washing steps are performed using PBS. The suppliers and catalogue numbers of each secondary antibody used are shown in blue.



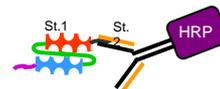
Immunocytochemistry (three stage labelling)

Primary antibody: PAM1_{scFv} (use at c. 20 µg/mL)
Secondary antibody: anti-HIS (1/100) [Sigma H-1029]
Tertiary antibody: anti-mouse/FITC (1/50) [Sigma F-6257]



Immuno-dot-assays (two stage detection)

Primary antibody: PAM1_{scFv} (use at c. 10 µg/mL)
Secondary antibody: anti-HIS/horse radish peroxidase (1/500) [Sigma A-7058]



References

- (1) Nissim *et al.* (1994) **EMBO J.** 13, 692-697
- (2) Willats *et al.* (1999) **Plant J.** 18, 57-65
- (3) Manfield *et al.* (2005) **Plant Science** 169, 1090-1095
- (4) Willats *et al.* (2000) **Carbohydr. Res.** 327, 309-320

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