

INDIRECT IMMUNO-FLUORESCENCE ASSAY

([Wright, 2002](#))

Introduction

This procedure is used to determine the location of glomalin on fungal structures, roots, soil particles, mesh, etc. Instead of using a color developer, a fluorescent tag (FITC) is added to the IgM antibody that, under certain wavelengths of light, produces a greenish color. Since this molecule is bound to the antibody, the location of color is an indirect assessment of the location of glomalin. A secondary antibody conjugation is not needed in this procedure, because the site specificity and intensity is not being used to determine concentration.

Materials

Small sieves (made from 10mm ID polyvinyl chloride or other plastic tubing with a 40 um nylon mesh glued to the bottom) or 40 um mesh bags to hold the sample ([Fig. 1](#))

2% non-fat milk (2 g powdered milk/100 ml PBS)

PBS (Phosphate buffered saline), pH 7.4

PBST (PBS with 0.2 ml/L Tween 20), pH 7.4

1% BSA (1 g BSA/100 ml PBS)*

MAb32B11 antibody (diluted with PBS, typically 1:1 or 1:2)**

FITC tagged goat anti-mouse IgM (24 ul/6 ml 1% BSA)**

VectaShield Mounting Media

Epi-fluorescence microscope with blue filter***

*Make about 500-1000 mls of stock and dispense in 6 ml aliquots that will be frozen until needed.

**These are recommended concentrations. Chemicals obtained from different companies or with different lot numbers may need to be optimized for your conditions. Optimize for ELISA and use the same concentrations in this assay.

***Use a microscope with a band pass combination BP450-BP490 excitor filter, a dichroic chromatic beam splitter FT-510 filter, and a longwave pass LP-520 barrier filter



Figure 1. Placing roots in sieves allows roots to be submerged in solution while easing replacement of solution by removing the sieves with forceps and inverting the plate.

Methods

- 1) Place sample in small sieve or mesh bag and place sieves or bags in wells or containers where the sample will be immersed in the liquid, but the amount of liquid needed is rather small.
- 2) Submerge in 2% milk and incubate, while shaking for 30 min.
- 3) Remove milk, add the diluted MAb32B11 antibody, and incubate on shaker for 1 hour.
- 4) Remove antibody, add PBST and incubate on shaker for 5 min. Repeat PBST incubation twice.
- 5) Add FITC tagged goat anti-mouse IgM and incubate on shaker for 1 hour. Remove IgM, add PBST, and incubate on shaker for 5 min. Repeat PBST incubation twice, followed by one 5-min incubation with PBS.
- 6) Develop with VectaShield mounting media and observe under [epi-fluorescence](#) using a blue filter ([Fig. 2](#)).

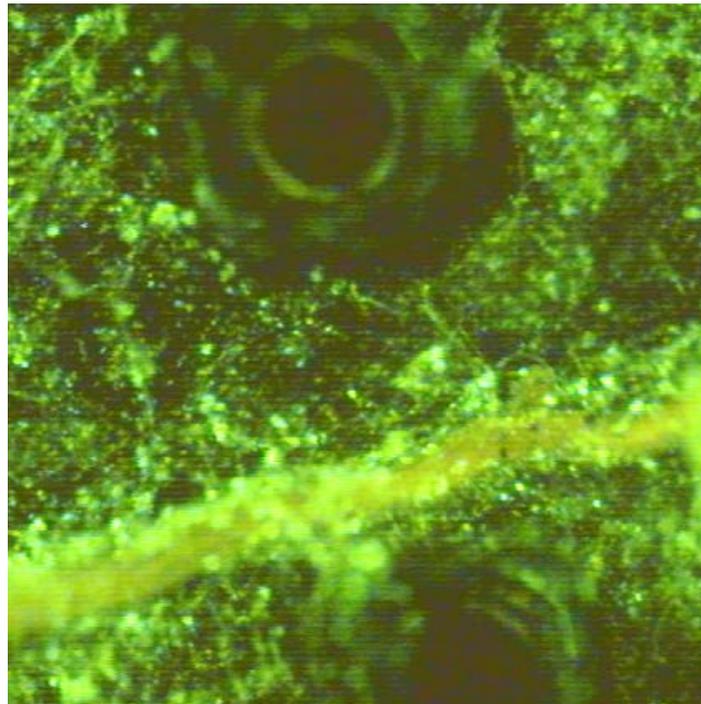


Figure 2. Bright green spots on the root, hyphae, and horticultural mesh indicate the presence of glomalin at these locations.

[Note: Immunofluorescence of intraradical structures requires a pre-incubation step with boiling 10% KOH for 10-15 min and a 2-hr incubation in 1:1 diluted MAb32B11 are advised for good color development. In addition, a counterstain, such as erichrome black, may be used prior to addition of the mounting medium.](#)