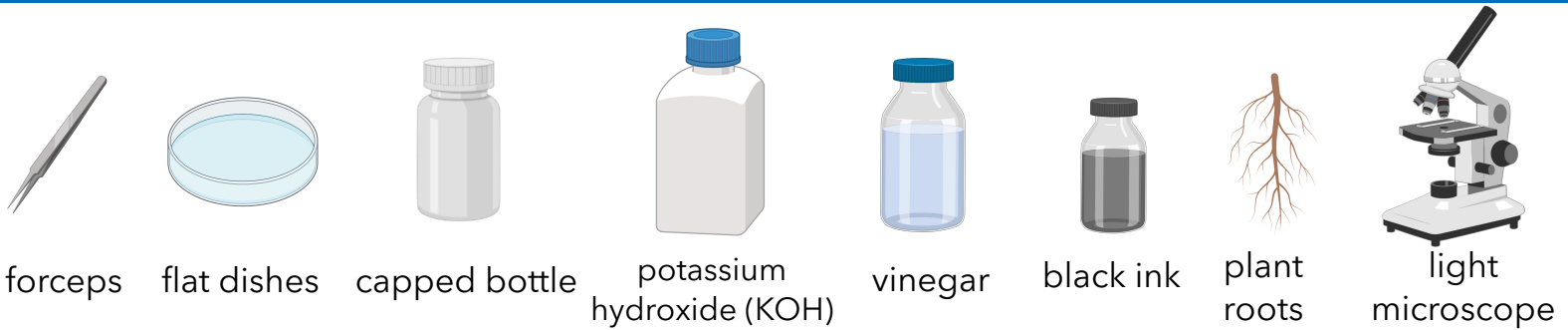


Protocol for Staining Plant Roots for Mycorrhizal Fungi

On-Farm Soil Biology Monitoring

Step 1: assemble required materials



Step 2: prepare the following solutions

- **10% KOH** (10 g KOH in 100 ml water) (*safety advice - avoid skin contact with KOH*)
- **5% vinegar** (5 mL vinegar + 95 mL water)
- **5% black ink** (5 mL black ink + 95 mL of 5% vinegar)

Step 3: prepare the roots for staining by clearing with KOH

- Collect and clean plant roots w/ tap water and cut into ~2 cm pieces.
- Place roots in bottle and cover with 10% KOH. Leave roots in 10% KOH for 5 to 7 days at room temperature.
- After roots are cleared by 10% KOH, rinse roots with water several times.

Step 4: stain the AMF in the roots

- Rinse roots with 5% vinegar one time.
- Add roots to 5% black ink and leave overnight.
- After the roots are stained by 5% black ink, rinse roots with water once.

Step 5: visualize the AMF roots

- Place the stained roots in flat dish and cover with water.
- View roots with light microscope.
- Root sample can be stored in water for several weeks.

Additional Resources

- (1) Protocol taken from [On Farm Soil Monitoring Handbook](#), a joint project led by Wheatbelt Natural Resource Management, the South West Catchments Council, and University of Western Australia.
- (2) Vierheilig et al. (1998) [Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi](#). Applied and Environmental Microbiology 64: 5004-5007.