Immunofluorescence
(Danforth Plant Science Center, Integrated Microscopy Facility)

- Titrate primary and secondary antibodies to determine the optimum working concentration--label cells of interest for this titration. Then store concentrated antibody (as acquired from vendor) in aliquots that will be diluted for the volume required for a given experiment. Avoid storing diluted antibodies and avoid freezing/thawing cycles. A good strategy is to dilute the vendor supply 50% with glycerol and freeze--stays semiliquid in the frozen state (dilute working solution accordingly). Check vendor’s instructions on storing the antibody.

- Multiple labeling can be simultaneous, if the primary antibodies are raised in different animals or sequential if they are raised in the same animal. In that case one set of primary and secondary antibodies is followed by the second set with a fixation step in between.

General protocol

1. fix in fresh, buffered (e.g., 50 mM PIPES pH 6.8) 4% paraformaldehyde for ~ one hour
2. rinse
3. permeabilize cells
4. rinse in PBS
5. block ~1 hour in blocking agent in PBS
6. rinse in PBS
7. incubate in primary antibody diluted in PBS for ~1 hour @RT or overnight at 4 degrees
8. rinse in PBS
9. incubate in secondary antibody diluted in PBS for 30-60 minutes
10. rinse in PBS
11. mount in antifade and seal
12. store in the dark at 4 degrees C overnight to let the antifade permeate the tissue
Additional tips

• Plant cell autofluorescence is more pervasive, especially chlorophyll (peak at 680 nm) and lignin (broad emission in the 460-560 nm range); choose the secondary accordingly.

• Permeabilize cells. The plant cell wall is a barrier to antibodies: after fixing digest in cell wall degrading enzymes (e.g., 1% cellulase plus 0.1% pectolyase) for 20-60 minutes (assess the right amount of time by monitoring in an inverted microscope). Additional treatment to permeabilize the cell membrane might be needed, such as treatment with dilute Triton (e.g., 0.1%).

• Root tip tissue is preferred rather than leaf tissue, which has a tough to remove cuticle.

• Blocking agent is typically 1% BSA plus 0.02% Tween-20 in PBS (PBST). Even more has been used, up to 20% fetal bovine serum albumin in PBST.

• Coat slides or coverslips with 0.1% PEI (polyethylenimine) to facilitate cell adherence. Use a PAP pen to draw wells if needed.