

Whole-mount immunolabeling of *Arabidopsis* seedlings

1. Fixation

4% paraformaldehyde in PBS + 0.1% Triton X-100

Incubate 1 hr in vacuum

2. Washing

2x10 min PSB

2x10 min dH₂O

3. Preparing slides

Transfer seedlings on Superfrost Plus slides, dry the liquid around the seedlings, cover with coverslip (don't press it too hard) and dip into the liquid nitrogen until it stops boiling. Remove the coverslip and let the slides dry completely (can be left overnight, but at least until all the liquid is gone). After they are dried, make circle with PapPen around the seedlings (not so necessary).

4. Rehydration

Rehydrate with PBS for few minutes (10-15 min).

5. Reduction (can be skipped)

NaBH₄ in PBS/Glycine

1mg/ml

PBS/Glycine

375 mg of glycine in 500 ml PBS

200 µl of solution per slide

Incubate 3x10 min

6. Washing

3x5 min with PBS/Glycine

7. Cell wall digestion

2% Driselase/PBS

Centrifuge the solution for 2 min

200 µl of solution per slide

Incubate 30 min at 37°C

8. Washing

4x5 min with PBS

9. Permeabilisation

10% DMSO/2% Nonidet P-40 in PBS

200 µl of solution per slide

Incubate for 1 hr at RT

10. Washing

4x10 min with PBS

11. Blocking

2% BSA in PBS

Incubate 1 hr at 37°C

12. Incubation with antibodies

1st antibody

Dilute in 2% BSA/PBS

200 µl of solution per slide

Incubate overnight at 37°C

Washing

6x10 min with PBS

2nd antibody

Dilute in 2% BSA/PBS

200 µl of solution per slide

Incubate for 3 hrs at 37°C

Washing

6x10 min with PBS

13. DAPI staining (not necessary)

Dilute the stock solution with PBS 1:1000

200 µl of solution per slide

Incubate 10 min at RT

14. Mounting

With p-phenylenediamine containing anti-fading medium