

Immunofluorescence labeling of root hairs

(*Zea*, *Arabidopsis*, *Medicago*)

Solutions:

MTSB

1000 ml dH₂O
15,1 g PIPES (disodium salt) (50 mM)
1,23 g MgSO₄ (5 mM)
10 ml EGTA stock solution (5 mM)
set pH to 6,9 with KOH

Note: For *Arabidopsis* and *Medicago* use ½ MTSB

EGTA stock solution

3,8 g EGTA in 10 ml H₂O, add solid KOH until clear, make to 20 ml, pH=8

Fixation solution

9,08 ml MTSB
520 µl formaldehyde (37 %) (=2 %)
400 µl glutaraldehyde (25 %) (=1 %)
set pH to 7,2 with KOH

PBS (10x stock sol.)

80 g NaCl
2 g KCl
11,5 g Na₂HPO₄
2 g KH₂PO₄
adjust to pH 7,3

PBS/Glycine

500 ml PBS (1x)
0,375 mg Glycine

Enzyme solution

30 mg Cellulase 0,3 %
30 mg Pectinase 0,3 %
5 mg Pectolyase 0,05 %
720 mg Manitol 0,4 M
10 ml PBS/Glycine

Incubation buffer

500 ml PBS (1x)
0,375 g Glycine (= 50mM)
0,1 % BSA
0,05 % Tween 20

Permeabilisation buffer

100 ml PBS (1x)
0,1 % Triton X-100
0,05 % Tween 20 (Nonidet P-40)

DAPI stock (100 mM)

1 mg in 10 H₂O

DAPI working solution

50 µl of 100 mM stock solution in 5 ml PBS

Mounting medium

100 mg p-phenylenediamine in 10 ml phosphate-saline (0,01 M phosphate buffer pH=7,4, 0,15 M NaCl), add 90 ml of glycerol

set pH to 8,0 with carbonate/bicarbonate or Tris pH=8,0

store at 0°C

Procedure:

1. Fixation

(cut samples into 2-4 mm small pieces and move them into fixation sol.) 30-60 min

2. Washing

MTSB 10 min

MTSB/PBS/Glycine (1:1) 5 min

PBS/Glycine 10 min

3. Permeabilisation

methanol (-20°C) 5 min

4. Washing

PBS/Glycine 2xshort

5. Enzyme digestion

enzyme sol. at 30°C for *Arabidopsis* 10 min

for *Medicago* 20 min

for *Zea* 30 min

6. Washing

PBS/Glycine 2x5 min

7. Permeabilisation

permeabilisation buffer 30-60 min

8. Samples preparation

Put some samples on the PEI (polyethyleneimine) (0,1 % in dH₂O) coated slides, dry them well, cover with another slide, squeeze a little bit, then immediately place this “sandwich” in liquid nitrogen (1-2 min), take it out, divide the slides and stick them together again, hit this “sandwich” few times so the tissue can be broken, let them defreeze and apply antibody solutions. Before applying the antibodies make a circle with wax pen around the area with samples.

9. First antibody

cca. 20-50 µl of first antibody in PBS/Glycine with 0,1% BSA at 37°C 2-3 h
at RT overnight

10. Washing

PBS/Glycine change 3x

11. Second antibody

cca. 20-50 µl of second antibody PBS/Glycine with 0,1% BSA (in dark room at 37°C) 2-3 h

12. Washing

PBS/Glycine change 3x

13. DAPI

cca. 20-50 µl of DAPI working solution 10 min

14. Washing

PBS/Glycine change 3x

15. Applying mountant medium

add 20-30 µl of mountant medium, cover with cover slides and seal with nail varnish

Note: The samples can be stored for 2-4 weeks at -20°C.