GUS histochemical staining

Note: The staining solution contains toxic chemicals. Handle it in the fume hood and dispose into special container. Wear gloves!

A. Prepare GUS staining solution

Substrate: X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide cyclohexylammonium salt). stock solution 20 mg/ml in dimethylformamide, keep at –20°C and wrapped with foil.
Other chemicals: Na_2HPO4, NaH_2PO4, Na_2EDTA, sodium ferrocyanide, sodium ferricyanide, Triton X-100, chloramphenicol, 70% ethanol

GUS-staining solution	Stock	For 200 ml	Final conc.
sodium phosphate buffer pH 7	0.2 M	100 ml	100 mM
EDTA	0.25 M	8 ml	10 mM
Triton X-100 [w/v]	20%	1 ml	0.1%
chloramphenicol	50 mg/ml	400 µl	100 µg/ml
potassium ferrocyanide	0.2 M	2 ml	2 mM
potassium ferricyanide	0.2 M	2 ml	2 mM
X-glucuronide (in N,N,formamide)	20 mg/ml	5 ml	0.5 mg/ml
		fill up to 200 ml with sterile water	

Filter-sterilize the solution and keep at 4°C (up to several months if kept dark).

B. Staining procedure

1. Put plant material to be stained into a tube (typically 15 ml falcon for staining of many seedlings) containing GUS staining solution. Press 2x2 cm square of fine nylon mesh into the tube to keep plants submerged during vacuum step.

2. Place open tubes in a dessicator and draw vacuum for 5-10 minutes for in vitro grown plants (up to 20 min for soil grown plants) to remove air and infiltrate the staining solution.

3. Release vacuum slowly, close the tube and incubate in the dark (incubator or wrapped in aluminum foil) at 37°C overnight.

4. Carefully remove the staining solution (nylon mesh usually keeps the material in the tube) and replace with 70% EtOH. Clearing is more rapid at 37°C, and several changes of EtOH may be necessary. The stained seedlings are stable for several months in ethanol at 4°C.

5. Examine stained seedlings in 70% ethanol under a stereomicroscope.