GUS staining

X-GlcA, buy from CyrusBioscience

> IUPAC name: 5-Bromo-4-chloro-1H-indol-3-yl β-D-glucopyranosiduronic acid
> Other name: 5-Bromo-4-chloro-3-indolyl-β-D-glucuronide
> Molucular formula: $C_{14}H_{12}BrClNO_7Na$
> Molecular weight: 444.6 g/mol

Procedure

Step 1: Prepare sodium phosphate buffer

50 mM, pH 7.0
Calculator

Chemical	M.W. (g/mol)	Addition	Storage location
NaH_2PO_4	119.98	249.0 mg	III
Na_2HPO_4	141.96	414.0 mg	III
ddH_2O		100 mL	

Step 2: Prepare X-GlcA stock solution (100 mM)

Dissolve 444.6 mg of X-GlcA in 10 mL **N,N-Dimethylformamide (DMF)** or DMSO.
I keep the stock solution in −20°C JK's white box. Must be **protected from light**.

Chemical	M.W. (g/mol)	Addition	Storage location
X-GlcA	444.60	444.6 mg	−20°C JK's white box
DMF		10 mL	Toxic (D)

Step 3: Prepare GUS stain working solution

The working solution must be fresh-made and use immediately after prepared.

> 1 mM X-Gluc
> 50 mM sodium phosphate buffer (Na-P) (pH 7.0)
> 7% methanol
> 0.1% TritonX-100

Chemical	Concentration	Addition	Storage location
X-GlcA stock solution[†]	100 mM	1 mL	−20°C
Methanol	100%	7 mL	Toxic (D)
TritonX-100	100%	1 drop	II
Sodium phosphate buffer (pH 7.0)	50 mM	92 mL	

[†] The stock solution is stored in the door compartment of the −20°C freezer in a black 10 mL tube.

Step 4: GUS Stain

1. Fully immersed the rice seedling in the GUS stain solution;
2. Vacumm infiltration at room temperature for 30 mins to allow the solution penetrate completely into the tissue;
3. Incubate at 37°C in dark for 30 mins;
4. (optional) Rinse with 75% or 95% ethanol until the chlorophyll was bleached;
5. Rinse with water to fully wash out the stain solution;
6. Observe under a dissection microscope.

Done !!!
