BIOLOGY

GUS expression analysis in Arabidopsis

Day 1

- Fix tissue in 90% acetone for 30 minutes.

Tissue can be kept in this solution for a few days at 4ºC

- Wash twice with water before GUS staining.
- Submerge the roots in the GUS staining solution:
  - 50mM Phosphate buffer pH=7
  - 0.1% Triton TX-100,
  - 1.5mM Potassium Ferrocyanide,
  - 1.5mM Potassium Ferricyanide
  - 2mM X-Gluc (5-bromo-4-chloro-3-indolyl ß-D-glucuronide cyclohexamine salt dissolved in DMSO – APOLO SCIENTIFIC - BIMB1021)

X-GLUC MOLECULAR WEIGHT: 521.79. ***==> use 10mg of X-Gluc disolved in 100uL of DMSO for each 10mL of solution.***

Stock	FINAL CONCENTRATION	FOR 10mL solution
Phosphate buffer (0.2M)	50mM	2.5mL
Triton TX-100 (10%)	0.1%	100uL
Potassium Ferrocyanide (0.1M)	1.5mM	150uL
Potassium Ferricyanide (0.1)	1-5mM	150uL

- Incubate at 37ºC in the dark for 18 hours.

Day 2

- Wash tissue with increasing concentrations of diluted ethanol (70%, 50%, 30% and 10%) and then water
- Mount the samples in Hoyer’s solution on microscope slides.
- The activity of the GUS reporter gene is then observed under the microscope.