GUS: Histochemical Staining with X-Gluc

A. Protocol:

1. Immerse tissue in staining solution. Microfuge tubes work well for small tissue samples; orange cap tubes work better for larger tissue samples.

2. Vacuum infiltrate briefly (not necessary for tissue culture grown Arabidopsis).

3. Incubate tissue overnight at 37°C.

4. Remove staining solution and wash with several changes of 50% ethanol until tissue clears. Incubate approximately 12 hours between each 50% EtOH change.

B. Solutions:

1. Staining solution (make fresh immediately prior to use).

	Concentration		
	Stock	Want	Per 1.0ml final volume
a. H_2O			830.0µl
b. $NaPO_4$ pH 7.0	1.0M	0.1M	100.0µl
c. EDTA	0.5M	10.0mM	20.0µl
d. Triton X-100`	10%	0.1%	10.0µl
e. $K_3Fe(CN)_6$	50mM	1.0mM	20.0µl
f. X-Gluc	0.1M	2.0mM	20.0µl

2. 50mM FerriCyanide ($K_3Fe(CN)_6$). Okay to store at RT but for long term; storage at –20°C is probably better. Discard if solution darkens.

	Concentration		
	Stock	Want	Per 5.0ml final volume
FerriCyanide ($K_3Fe(CN)_6$)	329.26 FW	50mM	82.3mg
H_2O			to 5.0ml

3. 0.1M X-Gluc (store at –80°C)

	Concentration		
	Stock	Want	Per 1.0ml final volume
X-Gluc	521.8 FW	0.1M	50mg
N,N-Dimethylformamide(N,N-DMF)			1.0ml

X-Gluc: 5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronide cyclohexylammonium salt, Gold Biotechnology (http://www.goldbio.com/open.htm) Catalog # G1281C ($68.00/100mg).

Reference:

Jefferson, R. (1987) Plant Mol. Biol. Reporter, 5:387-405.