**Using X-Gluc to visualize GUS activity**

(Reference: Ueli Grossniklaus, Cold Spring Harbor Laboratory)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Stock** | **10 ml** | **25 ml** | **50 ml** | **100 ml** | **Final Conc.** |
| 0.5M NaPO4 (pH 7) | 1 ml | 2.5 ml | 5 ml | 10 ml | 50 mM |
| 0.5M EDTA (pH 8) | 200 µl | 500 µl | 1 ml | 2 ml | 10 mM |
| 0.1M K4FeCN | 200 µl | 500 µl | 1 ml | 2 ml | 2 mM |
| 0.1M K3FeCN | 200 µl | 500 µl | 1 ml | 2 ml | 2 mM |
| Water | 6.4 ml | 16 ml | 32 ml | 66 ml |  |
| Methanol | 2 ml | 5 ml | 10 ml | 20 ml | 20 % |
| Triton X-100 | 10 ul | 25 ul | 50 ul | 100 ul | 1ul/ml |
| X-GLUC/DMSO | 5mg/25µl | 15mg/50µl | 25mg/125µl | 50mg/250µl | 0.5mg/ml |

1. Incubate at 37°C overnight or longer (usually 24 hrs), in the dark.

2. Destain by soaking in 1:1:3 *lactic acid:glycerol:PBS* several hours/days.

Note: can also destain in successive 20%, 35%, 50%, and 70% ethanol at room temperature for 30 minutes each, which destains faster and removes chlorophyll.

**Notes:**

1. Babu \_\_\_\_ at Iowa says staining of waxy tissues is much better if tissue is pretreated by a quick dip or wipe with chloroform which removes the wax.

2. Doug Darnowsky claims that there is less leakage of the blue moiety if one does not add a surfactant and if one incubates at room temperature. If you do it this way, incubate longer.

3. The methanol has inhibitory effects on native plant X-Gluc degrading enzymes so adding it reduces false positives.

4. Can store aliquots at -20°C or lower.

\*solutions on next page

**SOLUTIONS**

100ml of 0.5M NaPO4

57.6 ml 0.5M Na2HPO4 (pH 7) (see below)

21.2 ml 1M NaH2PO4 (pH 7) (see below)

21.2 ml water

 0.5M Na2HPO4 (pH 7)

 13.4g Na2HPO4•7H2O

 100mL Water

 1M NaH2PO4 (pH 7)

 13.8g NaH2PO4•1H20

 100mL Water

1X PBS (Maniatis recipe)

8g NaCl

0.2g KCl

1.44g Na2HPO4 pH to 7.4 with HCl

0.24g KH2PO4 Add H2O to 1000mls

800 ml H2O Filter sterilize (also removes lint)

0.1M K4FeCN

4.22g K4FeCN

100 mL water

0.1M K3FeCN

3.29g K3FeCN

100mL water

Lactic Acid: Sigma #L-1250 DL-Lactic Acid approx 98% (85% of which is synthetic).

X-Gluc: 5-bromo-4-chloro-3-indolyl glucuronide.