

## **Xgal stain**

	<u>25 ml</u>	<u>10 ml</u>
Xgal wash buffer	24.1 ml	9.64 ml
K <sub>3</sub> Fe(CN) <sub>6</sub>	41 mg	16.4 mg
K <sub>4</sub> Fe (CN) <sub>6</sub>	52.5 mg	21 mg
0.085 % NaCl	0.4 ml	0.16 ml
50 mg/ml Xgal in DMF	0.5 ml	0.2 ml

Filter with 0.2 µl filter. Store in dark at 37°C. If solution becomes contaminated, filter again. It is good for about two weeks.

## **0.2 % Glutaraldehyde**

10 ml:  
80 µl of 25% glutaraldehyde stock  
200 µl of 0.25 M EGTA (EGTA dissolves at high pH)  
9.7 ml 0.1 M phosphate buffer  
20 µl 1 M MgCl<sub>2</sub>

Phosphate buffer:

Make 0.1M solutions of monobasic and dibasic sodium phosphate:

Monobasic: 1.38 g in 100 ml dH<sub>2</sub>O

Dibasic: 2.68 g in 100 ml

\*Mix 5.75 ml monobasic with 19.25 ml dibasic to make 25 ml  
(or 28.75 ml monobasic with 96.25 ml dibasic to make 125 ml)

(Store in at 4°C, good for several weeks.)

## **Wash buffer:**

## **For 500 ml:**

0.1 M phosphate buffer (pH 7.3)	36.45 ml- 1.0M dibasic Na phosphate
	13.55 ml-1.0M monobasic phosphate
2 mM MgCl <sub>2</sub>	1 ml of 1M stock
0.1% sodium desoxycholate	5 ml of 10% stock
0.02% NP40	1 ml of 10% stock
0.05% BSA	5 µl of 1000x stock (10 mg/ml)

(Store buffer in cold room in a sterile bottle to prevent contamination)

## **To stain embryos:**

**Fix embryos in either 4% PFA or 0.2% glutaraldehyde for 20 min.**

**Wash two times (10 mins each) in wash buffer.**

**Put in xgal stain for a few hours or overnight depending on gene expression.**  
**Rinse with PBS (2 times)**  
**Place back in fixative overnight.**  
**If embedding, process soon.**