

Lac Z Staining Protocol Used for Gene trapping

Buffers and solutions needed.

A pH of 8.0 is required for the buffers in order to reduce the background staining in the EBs. All other differentiation assays can be stained using pH 7.0-8.0 buffers.

1. 0.1M Phosphate Buffer (pH 8.0)

932ml 0.1M Sodium phosphate dibasic
68ml 0.1M Sodium phosphate monobasic
1000ml

This should give a pH of 8.0 but verify with a pH indicator strip.

2. Fix solution

0.4ml 25% gluteraldehyde
0.5ml 0.5M EGTA pH 7.3
0.1ml 1M magnesium chloride
49ml 0.1M sodium phosphate buffer
50ml

To be prepared fresh each time.

3. Wash buffer

0.4ml 1M magnesium chloride
2.0ml 2% Nonidet-P40
197.6ml 0.1M sodium phosphate buffer
200ml

4. X-gal stain

2.0ml 25mg/ml X-gal stock dissolved in di-methyl formamide
0.106g potassium ferrocyanide from Sigma (P-9387)
0.082g potassium ferricyanide from Sigma (P-8131)
48.0ml wash buffer
50.0ml

This stain can be stored at 4°C in the dark but should not be kept for more than one week.

Staining procedure for EBs

- Rinse cells once in phosphate buffer at room temperature
- Fix cells for five minutes at room temperature, completely covering cells with fix.
- Wash the cells three times for five minutes with wash buffer at room temperature.
- Add enough stain to completely cover the cells
 - 96 well, at least 80ul
 - 24 well, at least 300ul
 - 6 well, use 1ml
- Stain overnight at 37 degrees, remove stain, rinse with PBS or wash buffer and then replace with wash buffer. Store samples at 4°C. Staining will intensify in wash buffer with time at 4°C. 60% glycerol (in PSB) can be used to replace the wash buffer for long term storage at 4°C.