

Staining for beta-galactosidase activity

Solutions

PBS

0.05% glutaraldehyde: Dilute 25% stock 500x in PBS. Stock should be stored frozen at -20C in aliquots

X-gal solution: Prepare in PBS 30 mM potassium ferricyanide ($K_3Fe(CN)_6$), 30 mM potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$), 1 mM $MgCl_2$ or $MgSO_4$. Add Xgal to 1 mg/ml final concentration just before use. The solution minus the Xgal can be stored a few months at room temp.

Procedure

- 1.** Discard medium and wash twice with PBS. Add 2 ml fix (0.05% glutaraldehyde) per 60 mm dish and incubate for at least 5 minutes and no longer than 15 minutes.
- 2.** Rinse cells three times in PBS as follows: Wash 1 and 3 are quick washes. Wash 2 is 10 minutes.
- 3.** Add X-gal solution. Use a minimal volume to cover the cells, about 0.5 ml for a 60 mm dish.
- 4.** Incubate from 1 hr to overnight at 37°C. Positive cells will stain blue.
- 5.** Remove stain and overlay with 80% glycerol. The dishes can be stored indefinitely at 4°C