

## Beta-Gal Staining in Primary Neurons

(Z. Xia - 4-8-94)

1. Fix cells w/ 4 ml fix soln/10cm or 60 cm dish, 37°C, 5 min
  - Fix soln.: for 100 ml (use w/i 1 wk)
    - 5.4 ml 37% formaldehyde (final 2%)
    - 800 ul 25% glutaraldehyde (final 0.2%)
    - 94.5 ml PBS
2. Wash cells 1x w/ PBS prewarmed to 37°C
3. Stain w/2 ml staining soln./60 mm plate for 30 min to o/n. Blue cells may start to appear in 30 min.
  - Stain soln.:
    - Part a. 5 mM potassium ferricyanide ( $K_3Fe(CN)_6$ )
    - 5 mM potassium ferrocyanide ( $K_4Fe(CN)_6 \cdot 3H_2O$ )
    - 2 mM  $MgCl_2$
    - Nn PBS. Store @ R.T., use w/i 1 month or until see ppt.

For 200 ml of a.: 0.33 g  $K_3Fe(CN)_6$   
0.42 g  $K_4Fe(CN)_6 \cdot 3H_2O$   
0.4 ml 1M  $MgCl_2$

    - Part b. X-gal (5-bromo-4-chloro-3-indolyl-beta-gal)
    - 40 mg/ml in DMSO
  - Heat part a and b to 37°C.
  - Add part b to part a to final 1 mg/ml X-gal. Add part b slowly while mixing, or crystals will form.
  - Filter to remove crystals.
4. Remove stain soln, either replace w/ fixer and store @ 4°C, or wash w/ PBS and mount.