X-Gal Staining for Expression of LacZ in Cultured Cells

1. Aspirate off culture media and wash cells 1x with cold PBS

2. Fix the cells on ice with ~5mL glutaraldehyde (1:100 dilution of stock in PBS) for 5 min.

3. Rinse the cells 3x with PBS for 4 min. per wash.

4. Dilute 25x stock of X-Gal into the staining solution (final 1mg/mL of X-gal)

5. Add ~5mL of the X-gal staining solution to the cells and incubate at 37°C for 1-20 hours, depending on staining intensity. Cells should be checked every hour or so early in the stain to determine whether cells are turning blue.

Note: Do not put the staining cells into a tissue culture incubator, as the CO2 in the incubator will change the pH of the staining reaction and stop LacZ activity.

Stain Solution (store at 4°C with foil to keep dark)

- 50ug MgCl2
- 41 mg potassium-ferricyanide
- 53 mg potassium-ferrocyanide
- into 25mL of PBS (pH 7.3)