## 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  $CO_2$  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 MgCl<sub>2</sub>
- 41 mg potassium-ferricyanide
- 53 mg potassium-ferrocyanide
- into 25mL of PBS (pH 7.3)