# BETA-GALACTOSIDASE (X-GAL) STAINING ASSAY

Adapted from BV's Cookbook by TCH 8/18/01

**To stain T-25 flasks:**

1. Remove media from cells. (OPTIONAL: wash the cells with PBS).
2. Add 3ml fixative per T25 and incubate at room temp for 5 min.
3. Remove fixative. (OPTIONAL: rinse with PBS x 2, 2 ml each).
4. (Optional: Add 1ml X-gal stain and remove).
5. Add 2 ml X-gal stain, place cells at 37°C for 30 min to several hours.
6. Cells expressing β-gal will turn blue. Remove green filter from microscope to visualize.

**To stain 24 well plates:**

1. Remove media from cells. (OPTIONAL: wash the cells with PBS).
2. Add 1.0 ml fixative/well and incubate at room temp. for 5 min.
3. Remove fixative. (OPTIONAL: rinse with PBS, 2 x 1 ml).
4..Add 0.5 ml X-gal stain, place cells at 37°C for 30 min to several hours.

**Materials and Reagents**

**Fixative:** 0.05 % glutaraldehyde in PBS (for 10 ml, add 20 ul 25 % glutaraldehyde from Sigma to 10 ml PBS).

**X-gal stain** (prepare just before use)**:**

| Volume for 5 ml | Final Concentration |
|---|---|
| 500 ul 1 M $NaPO_4$ pH 7.3 | 100 mM |
| 300 ul 50 mM $K_3Fe(CN)6$ | 3 mM |
| 300 ul 50 mM $K_4Fe(CN)_6$ | 3 mM |
| 6.5 ul 1 M $MgCl_2$ | 1.3mM |
| 3.6 ml H2O | |
| 250 ul X-gal | 1 mg/ml |

- X-gal is kept at 20mg/ml stock in dimethylformamide at -20°
- Potassium ferricyanide stocks are stored at 4°