**β- GALACTOSIDASE ASSAY**

The recommended amount of RSV-β-Galactosidase plasmid to use for transfection of cells (60 mm or 100 mm dish) is 1-2 µg. The optimal amount of plasmid DNA will be determined by the efficiency of transfection, which is very dependent upon the particular cell line and transfection protocol.

β-Galactosidase assay 2x buffer:
- 200 mM sodium phosphate, pH 7.3
- 2 mM MgCl₂
- 100 mM β-mercaptoethanol
- 1.33 mg/ml ONPG (o-nitrophenyl-b-D-galactopyranoside)

1. Prepare the following reaction mixtures in microcentrifuge tubes:

   **Control**
   - β-galactosidase assay 2x buffer 150 µl
   - non-transfected cell extract 12.5 µg
   - ddH₂O to final volume of 300 µl

   **Sample Reaction**
   - β-galactosidase assay 2x buffer 150 µl
   - transfected cell extract 12.5 µg
   - ddH₂O to final volume 300 µl

2. Mix all samples by vortexing.

3. Incubate the reaction at 37°C for a fixed period of time until a yellow color is present, usually within 30 minutes. The incubations may be performed as long as 3 hours if the reaction tubes are tightly capped.

4. Stop the reaction by adding 500 µl of 1M sodium carbonate. Mix by vortexing.

5. Read the absorbance at 420nm.