ß- GALACTOSIDASE ASSAY

The recommended amount of RSV-ß-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.

-ß-Galactosidase assay 2x buffer:

200 mM sodium phosphate, pH 7.3
2 mM $MgCl_2$
100 mM ß-mercaptoethanol
1.33 mg/ml ONPG (o-nitrophenyl-b-D-galactopyranoside)

1. Prepare the following reaction mixtures in microcentrifuge tubes:

Control

ß-galactosidase assay 2x buffer	150 µl
non-transfected cell extract	12.5 µg
ddH_2O	to final volume of 300 µl

Sample Reaction

ß-galactosidase assay 2x buffer	150µl
transfected cell extract	12.5µg
ddH_2O	to final volume 300µl

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.