

## $\beta$ -Galactosidase Assay

Created: April 4, 1994  
Last Revision: October 24, 2012

1. Grow bacteria at desired temperature for desired time and mix the following:

	for 10 ml tubes	for microtitreplate
Sample	0.2 ml	10 $\mu$ l
Z buffer	2.8 ml	140 $\mu$ l
Toluene (or Acetone/Toluene 9:1)	20 $\mu$ l	1 $\mu$ l
Warm to 37°C, than add:		
ONPG (4 mg/ml)	0.4 ml	20 $\mu$ l

2. Incubate at 37°C until you see a color change to yellow; then add 1 M Na<sub>2</sub>CO<sub>3</sub> to stop reaction (1 ml for 10 ml tubes; 50  $\mu$ l for microtitreplates).

3. Read OD in spectrophotometer at 450 nm.

### BUFFERS:

Z buffer:

0.06 M Na<sub>2</sub>HPO<sub>4</sub>  
0.04 m NaH<sub>2</sub>PO<sub>4</sub>  
0.01 M KCl  
0.001 M MgSO<sub>4</sub>  
0.01 M  $\beta$ -mercaptoethanol