

# Reporter Assays for Luciferase and $\beta$ -Galactosidase

## Materials

### EGTA 0.1 M pH 7.8

EGTA (Sigma E-4378)	3.8 g
H <sub>2</sub> O	70 ml
pH to 7.8 with conc. NaOH (it dissolves only when pH is 7.8)	
H <sub>2</sub> O to final vol.	100 ml

### TRIS.PO4 1M pH 7.8

Trizma-PO4 dibasic (Sigma T4258)	17g
H <sub>2</sub> O	30 ml
pH to 7.8 with conc NaOH	
H <sub>2</sub> O to final vol.	50 ml

### CDTA 0.2 M

CDTA (Sigma D8928)	3.644 g
H <sub>2</sub> O	30 ml
pH to 7.8 with NaOH to dissolve	
H <sub>2</sub> O to final vol.	50 ml

### ATP 100 mM

ATP (Sigma A3377)  
Aliquot and freeze at -20

### DTT 1M

DTT (Sigma D9779)	1.549 g
H <sub>2</sub> O to final vol.	10 ml
Aliquot and freeze at -20	

## **Lysis Buffer**

1 M TrisPO4 pH7.8	12.5 ml
0.2 M CDTA	5 ml
Glycerol	50 ml
Triton-X 100	5 ml
H <sub>2</sub> O to final vol.	500 ml

Before use, add 10  $\mu$ l of 1 M DTT to 5 ml lysis buffer

## **Luciferase assay buffer**

1 M Glycyl glycine	12.5 ml
1 M MgSO <sub>4</sub>	7.5 ml
1 M KPO <sub>4</sub> pH 7.8	7.5 ml
0.1 M EGTA pH 7.8	20 ml
H <sub>2</sub> O to final vol.	500 ml

Note - I have left out the glycyl glycine without ill effect!

Before use, add: 200  $\mu$ l of 100 mM ATP  
20  $\mu$ l of 1 M DTT to 10 ml of assay buffer.

### Luciferin stock solution

D-Luciferin (Promega or Biosynth) 0.3 mg/ml in H<sub>2</sub>O  
Aliquots of 10 ml each, cover tubes in metal foil, and freeze at -20 degC

### Z Buffer

Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O	16.1g
(or Na <sub>2</sub> HPO <sub>4</sub> )	8.53g)
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	5.5g
1M KCl	10 ml
1M MgSO <sub>4</sub>	1ml
H <sub>2</sub> O to	1L

pH should be 6.95

Add 27 µl BME, and 1 ml 10x ONPG per 10 ml before use.

### 10 X ONPG

ONPG (Sigma) at 20 mg/ml as follows

1M Na <sub>2</sub> HPO <sub>4</sub>	600 ul
1M NaH <sub>2</sub> PO <sub>4</sub>	400 ul
H <sub>2</sub> O to	10 ml
ONPG	200 mg
Dissolve and freeze at -20 degC in 1 ml aliquots.	

### Na<sub>2</sub>CO<sub>3</sub> 1M

Na <sub>2</sub> CO <sub>3</sub>	53g
H <sub>2</sub> O to final vol.	500 ml

### EXPERIMENTAL PROTOCOL

Transfections, luciferase and lacZ in 24 well plates

**1.** Plate cells out the day before at a density of 5 X 10<sup>4</sup> cells/ml, 500 µl per well. The cell density should be kept constant between repeat experiments to get reproducible results. Cells should be **in log phase** at time of plating. Optimise the cell density for your cell type.

**2.** A typical transfection mix for a single well is:

Luciferase reporter	150 - 300 ng
pCMV-lacZ	50 - 100 ng
10% Fugene 6	5 - 10 µl

Dilute the Fugene 6 to 10% in serum free medium, wait 5 min, then add to the DNA mix in an Eppendorf tube, mix by pipetting. Wait 15 min, then add to the well.

Note that you should always have at least 1 µl of Fugene 6 per 0.5 µg of DNA, so if using more total DNA, use more 10% Fugene 6.

**3.** Harvest cells 24-48 hours later.

1. Remove media from plate, rinse 1x with PBS and aspirate.
2. Add 200 µl of lysis buffer per well.

3. Keep at RT on a shaker for 20 min, then transfer to ice. You can freeze it at this point at  $-20$ .

#### 4. Luciferase assays.

Use a luminometer with a dual injector system. Place luciferin solution to one injector (set to deliver  $50\ \mu\text{l}$  /  $150\ \mu\text{l}$ ), place luciferase assay buffer to other injector, (set to deliver  $100\ \mu\text{l}$  /  $300\ \mu\text{l}$ ).

Add 5 -  $10\ \mu\text{l}$  of the cell lysate per well of plate reader, or upto  $20\ \mu\text{l}$  per tube of tube reader.

#### 5. Lac Z assays

A multi channel pipetter helps here!

Pipette 5- $10\ \mu\text{l}$  of cell lysate per well of a flat bottomed ELISA plate

Add LacZ buffer  $100\ \mu\text{l}$

Wait. (10 min - 3 hrs depending on transfection efficiency)

Once colour is faint yellow, add  $50\ \mu\text{l}$  1M  $\text{Na}_2\text{CO}_3$  per well, color will become darker.

Read at A420. **Note that OD should not exceed 0.6 in any well as it is non-linear.**

You can set up a standard curve with lacZ purchased from Sigma.