

Luciferase assay

XL Yang, Jan. 15, 2002

A. Preparing cell extract

1. Wash the culture dish by 5 ml of PBS (-) three times
2. Add 1 ml of cooled EXTRACT BUFFER* into the dish
3. Rub the cell by a rubber policeman
4. Collect the cellular suspension into a Eppendorf tube
5. Centrifuge at 3,000 rpm for 3 min
6. Dispose the supernatant, add 100µl of EXTRACT BUFFER* and vortex
7. Freeze (-80°C, >20 min) and thaw (37°C, 1-2 min) 3 times
8. Spin at 15,000 rpm at 4°C for 5 min
9. Collect the supernatant into another new tube
10. Store at -80°C if not doing assay

B. Luciferase assay

1. Preparing samples in a Eppendorf tube at RT

ASSAY BUFFER**	150 µl
Cell extract	x µl (<50 µl)
EXTRACT BUFFER*	(50-x) µl

	200 µl

2. Transfer the sample to the special tube for the machine, then add 100µl of LUCIFERIN*** and mix them as soon, then put the tube into the luminometer followed by pushing the start key.

Note: The transfectional efficiency should be normalized by dividing the luciferase activity by the β -galactosidase activity (co-transfection of *LacZ* plasmid should be carried out).

Reagents:

***EXTRACT BUFFER**

0.1M potassium phosphate (pH 7.8)

1 mM DTT

**** ASSAY BUFFER**

100 mM k- Hepes pH 7.9 0.5 ml (1M, pH 7.9)

30 mM Mg acetate 0.15 ml (1 M)

1 mM DTT 5 μ l (1 M)

8 mM ATP 0.4 ml (100 mM)

H₂O 3.95 ml

5.00 ml (for 33 samples, make before use)

*****LUCIFERIN (Sigma, L-6882)**

2 mg in 3.3 ml H₂O