

β -Galactosidase 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 100 μ l 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. β -galactosidase assay

1. Preparing samples in Eppendorf tube at RT

100X Mg solution*	3 μ l
1X ONPG**	66 μ l
Cell extract	30 μ l
0.1 M sodium phosphate***	201 μ l

300 μ l

2. Incubate the reaction at 37°C for 30 min (or until a faint yellow color has developed)

3. Stop the reaction by adding 500µl of 1M Na₂CO₃ to each reaction
4. Read the optical density of the reaction at a wavelength of 420 nm (the linear range is 0.2-0.8 OD)

Reagents:

*100X Mg solution

0.1M MgCl₂

4.5M β-mercaptoethanol

**1X ONPG (o-nitrophenyl-β-D-galactopyranoside, Sigma N1127)

4 mg/ml in 0.1M sodium phosphate (pH 7.5)

***0.1M sodium phosphate

0.2M Na₂HPO₄·2H₂O 41 ml

0.2M NaH₂PO₄·2H₂O 9 ml

H₂O 50 ml

100 ml

Note:

It is essential to include positive and negative controls of the following type of β-galactosidase assays:

	Positive control (µl)	Negative control (µl)
Mg buffer	3	3
1X ONPG	66	66
β-galactosidase (50 units/ml)^{&}	1	0
Cell extract	30	30
0.1M sodium phosphate	201	201

&Dissolve *E. coli* β -galactosidase of 3000 units/ml in 0.1M sodium phosphate (pH 7.5). Just before use, transfer 1 μ l of the stock solution of β -galactosidase into 60 μ l of 0.1 M sodium phosphate. Use 1 μ l of the diluted stock (50 units/ml) for the positive control.