

Assay Method

1. Prepare o/n culture in Minimal media / L Broth (5ml in 25ml flasks)
Shake at 30°C overnight for assays with Mel promoter and Mel R.
2. Also prepare another identical set of flasks containing media and store overnight at 4°C. For assays with/without melibiose prepare two sets of flasks, one with melibiose added and one without melibiose.
3. The following day inoculate 150µl of overnight culture into 5ml of Minimal Media / L Broth in 25ml flasks and shake at 30°C.
4. Prepare required volume of ONPG in Z buffer and place in 37°C oven.
5. Set waterbath to 37°C.
6. Grow cultures up to 0.3A – 0.5A at 650nm.
7. Record absorbance of each sample.
8. Lyse remainder of sample (2ml) with one drop of toluene and one drop of sodium deoxycholate.
9. Vortex and aerate at 37°C for 20mins.
10. Pipette 100µl of samples into assay tubes including a blank of media.
11. At timed intervals add 2.5ml of ONPG solution.
12. Place the tubes in the waterbath at 37°C and leave until a yellow colour develops.
13. Stop the reaction by adding 1.0ml of 1M sodium carbonate, making a note of the time at which it is added.
14. Read the absorbance of the samples at 420nm using the blank to zero the instrument.

Lac Z assay reagents

Z buffer (1 litre) *

KCl	0.75g	
MgSO ₄ ·7H ₂ O	0.25g	
Na ₂ HPO ₄	8.53g	
NaH ₂ PO ₄ ·2H ₂ O	4.87g	
β-mercaptoethanol	2.70ml	(add before use) ^{500 μl in 200ml}
H ₂ O	up to 1 litre	

ONPG (13 mM)

100mg in 125ml Z buffer (add before use)
160mg in 200ml

Sodium Carbonate (1M)

Sodium Deoxycholate (1%)

Minimal Media

To prepare 100ml of media mix the following reagents:

M9 salts x10	10.0ml
Water	90.0ml
1M MgSO ₄	200 μl
0.1M CaCl ₂	100 μl
20% Fructose	1.5ml
20% Casamino acids	0.5ml

*
Make up Z buffer with no β-mercaptoethanol.
When you make up ONPG solution add β-Mercaptoethanol to the aliquot you use.
Eg. for 125ml of ONPG
125ml Z-buffer
100mg ONPG
338 μl of β-Mercaptoethanol

100 ml Z-buffer
27 μl β-mercapto
80 mg ONPG.

If required add the following:

40mg/ml Ampicillin	200 μl
10mg/ml Tetracycline	350 μl
25mg/ml Kanamycin	100 μl
20mg/ml Proline	100 μl
Aro 4	1.0ml
20% Melibiose	1.5ml

1 Lit. + Na₂HPO₄ → 60g
KH₂PO₄ → 30g
NaCl → 5g
NH₄Cl → 10g
↓
pH = 7.4 & autoclave
(final conc. = 0.3%)

add β-mercapto last!

* Cells grown overnight i.e. that were in stationary phase were also assayed.

$$\begin{aligned} \text{Moles} &= C \times V (30\text{ml}) \\ &= 1\text{M} \times (0.03) \\ &= 0.03 \end{aligned}$$

$$\begin{aligned} \text{Mass} &= \text{mm} \times \text{Mole} \\ &= 246.48 \times 0.03 \\ &= \end{aligned}$$