Beta-galactosidase activity assay for yeast

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Abstract

Beta galactosidase enzymatic activity assay for yeast

Reagents

- Z Buffer: 60 mM Na₂HPO₄, 40 mM NaH₂PO₄·H₂O, 10 mM KCl, 1 mM MgSO₄, pH 7 - β-mercaptoethanol (161-0710, Bio-Rad) - Zymolyase-20T (120491, SEIKAGAKU CORPORATION) - Protein Assay Dye Reagent Concentrate (500-0006, BIO-RAD) for Bradford Assay - ONPG (N1127, SIGMA ALDRICH)

Procedure

1. Collect approximately 5×10^7 cells from yeast exponentially growing cultures 2. Pellet cells by centrifugation at 4000xg at 4°C and wash once with water 3. Cells can be stored at -20°C 4. Resuspend cells with 200 µl of Z Buffer + β-mercaptoethanol (0.27% (v/v)) containing 1mg/mL zymolyase-20T 5. Incubate samples 20-30 min at 30°C to digest cells 6. Centrifuge cells at 13400xg for 5 min at 4°C 7. Transfer the supernatant to a new eppendorf 8. Use 1 or 2 µL of the extract to quantify the amount of protein using the Bradford assay (Concentration) 9. Mix 180 µl of extract (Volume) with 700 µL of Z Buffer + β-mercaptoethanol 10. Start the reaction by adding 200 µL of 4mg/mL ONPG (o-nitrophenyl- β-D-galactopiranoside 11. Incubate samples at 30°C until they become yellow coloured (up to 90 minutes) 12. When yellow colour appears (Time), stop the reaction with 500 µL of Na₂CO₃ 1M 13. Centrifuge cells at 13400xg for 1 min to pellet cells 14. Measure A₄₆₀ 15. β-galactosidase activity is expressed in arbitrary units (U), from the following formula:
$$U = \frac{(A_{460} \times 1000)}{(Time \text{ (min)} \times Volume \text{ (µL)} \times Concentration \text{ (µg/µL)})}$$