untitled protocol

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Abstract

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Materials

- Nitrophenyl b-d-galactopyranoside View by P212121
- Magnesium Sulfate (MgSO4) Solution - 6.0 ml B1003S by New England Biolabs
- Sodium Phosphate monobasic by Contributed by users
- Sodium phosphate dibasic 7558-79-4 by Sigma Aldrich
- 500g Sodium Carbonate; Na2CO3 (anhydrous) RC-125 by G-Biosciences
- KCl by Contributed by users
- 1% β-mercaptoethanol by BBI Biotech

Protocol

Exponentially growing cells (1 ml) at 37°C in ABTGcasa medium were collected at OD450=0.1, 0.2, 0.3, 0.4 and 0.5

Step 1.

Mix with cold toluene (0.1 ml) and kept on ice immediately

Step 2.

0.2 ml toluene-treated sample was added to 1 ml Z buffer (40 mM NaH_2PO_4, 60 mM Na_2HPO_4, 10 mM KCl, 1 mM $MgSO_4$ and 50 mM β-mercaptoethanol, pH 7.0) containing 0.66 mg/ml o-nitrophenyl-β-D-galactopyranoside.

Step 3.

The reaction was performed at 30°C until the color changed to yellow

Step 4.

Stop by addition of 0.5 ml 1 M Na_2CO_3

Step 5.

The absorbance at OD420 was measured

Step 6.

The β-galactosidase activity was calculated by 1000×OD420/reaction time (min) ×OD450×0.2 ml

Step 7.