

Bacteria Culture Protocol

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1、 TBS Medium Preparation:

Prepare 1L of TBS medium contains:

Tryptone	12g
Yeast extract	24g
NaCl	5g
Sodium Succinate	5g
Glycerol	20ml

Adjust PH to 7.0 after totally solved.

Sterilize with 121°C, 15min. When medium Tm is below 60°C, add 1/1000 Glucose&MgSO₄ store solution (Dissolve 25g Glucose and 12.3g MgSO₄ • 7H₂O in ddwater to a final volume of 50ml, then sterile by filtered with a 0.22um membrane).

2、 TBS Medium Culture Protocol (for DH5a)

Inoculate a single clone to 50ml TBS medium in a 250ml baffled flask . Incubate in 250rpm/37 °C for 24h, Be sure that air inner the flask can exchange well with outside.

3、 Auto-induction Medium (AIM) Preparation (for BL21de3)

Prepare 1L of AIM contains:

Tryptone	12g
Yeast extract	24g
Sodium Succinate	4g
Sodium Citrate	6g
Glycerol	20ml
Lactose	5g

Adjust PH to 7.0 after totally solved.

Sterilize with 121°C, 15min. When medium Tm is below 60°C, add 1/1000 Glucose&MgSO₄ store solution (Dissolve 25g Glucose and 12.3g MgSO₄ • 7H₂O in ddwater to a final volume of 50ml, then sterilize by filtered with a 0.22um membrane).

4、 Auto-induction Medium (AIM) Expression Method

Inoculate a single clone to 50ml TBS medium in a 250ml baffled flask. Incubate in 250rpm/37 °C for 6h, then change Tm to your induction Tm and continue culturing until OD600 reaches 20-40 for full induction. Be sure that air inner the flask can exchange well with outside.

Note: Collect bacterium in a slightly lower centrifuge speed to prevent from contaminated by medium ingredients or metabolites, we recommend 3000rpm for J6-MI (Backman).