Freezing protocol for Escherichia coli

Andreas Sagen[1]

[1]University of Oslo

Andreas Sagen
University of Oslo, The National Institute of Occupational H...

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ABSTRACT

An optimized freezing protocol of E. coli based on Zimmer and Verrinder Gibbins 1997.

Protocol status: In development
We are still developing and optimizing this protocol

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Preparation of LB-Lennox

1 Add 800 mL distilled water to a Duran flask

2 Measure 10 g tryptone, 5 g yeast extract and 5 g sodium chloride

Materials:
Tryptone **Millipore Catalog #T9410**
Yeast Extract **Sigma-aldrich Catalog #Y0875**
Sodium chloride **Sigma-aldrich Catalog #S9625**

3 Adjust pH to 7.0 with sodium hydroxide

4 Fill distilled water to a total of 1000 mL

5 Sterilize by autoclave at 121 °C for 00:15:00 15m

Preparation of LB Freezing Buffer

6 Fill 80 mL LB-Lennox to a Duran bottle

7 Add 1.32 mL Potassium phosphate monobasic ([M] 1 Molarity (M)), 3.6 mL Potassium phosphate dibasic ([M] 1 Molarity (M)), 50 µL magnesium sulfate ([M] 1 Molarity (M)), 170 µL sodium citrate ([M] 1 Molarity (M)), 680 µL ammonium sulfate ([M] 1 Molarity (M)) and 4.4 mL glycerol.

> Note
>
> Remember to sterilize all components before they are combined aseptically.

Materials:

Potassium phosphate dibasic **Sigma-aldrich Catalog #P3786**
Potassium phosphate monobasic **Sigma-aldrich Catalog #P5379**
Magnesium sulfate **Sigma-aldrich Catalog #M7506**
Sodium citrate tribasic dihydrate **Sigma-aldrich Catalog #S4641**
Ammonium sulfate **Sigma-aldrich Catalog #A4418**
Glycerol **MP Biomedicals Catalog #194680**

8 Dissolve salts by stirring

9 Adjust pH to pH 7.0

10 Fill to 100 mL with LB-Lennox

Freezing procedure

5m

11 Transfer a saturated culture of E. coli cells

> **Note**
>
> It is important that the bacteria culture is saturated, but not oversaturated. An OD_{600} between 0.4 and 0.6 is ideal.

12 Centrifuge at 1000 rcf, 00:05:00 5m

13 Remove supernatant, and resuspend pellet in an 10% the initial volume

14 Aliquot saturated culture into an appropriate container. For example:

100 µL saturated culture in a 200 µL reaction tube

1000 µL saturated culture in a 1.5 mL reaction tube

1500 µL saturated culture in a 2.0 mL cryotube

15 Transfer to an ultra-low temperature or vapor-phase nitrogen freezer for long-term storage

> **Note**
>
> If possible, snap-freeze tubes in liquid nitrogen.