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

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Freezing protocol for Escherichia coli

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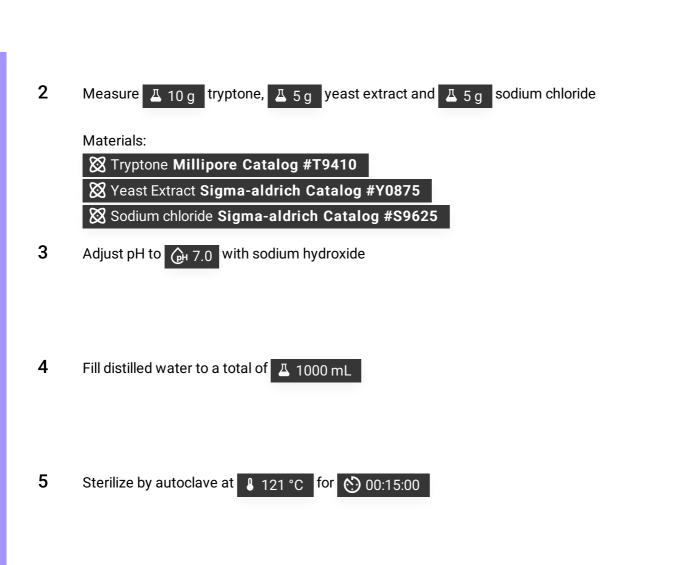
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ABSTRACT

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

Preparation of LB-Lennox

Add 🛚 800 mL distilled water to a Duran flask



Preparation of LB Freezing Buffer

- 6 Fill A 80 mL LB-Lennox to a Duran bottle
- Add Δ 1.32 mL Potassium phosphate monobasic ([M] 1 Molarity (M)), Δ 3.6 mL Potassium phosphate dibasic ([M] 1 Molarity (M)), Δ 50 μL magnesium sulfate ([M] 1 Molarity (M)), Δ 170 μL sodium citrate ([M] 1 Molarity (M)), Δ 680 μL ammonium sulfate ([M] 1 Molarity (M)) and Δ 4.4 mL glycerol.

Note

Remember to sterilize all components before they are combined aseptically.

Materials:

15m

- Sodium citrate tribasic dihydrate Sigma-aldrich Catalog #S4641
- **⊠** Glycerol **MP Biomedicals Catalog #194680**
- **8** Dissolve salts by stirring
- Adjust pH to PH 7.0
- 10 Fill to 4 100 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 (3) 1000 rcf, 00:05:00

5m

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

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

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.