

Sep 26, 2019

Preparation of Chemically Competent Cells

DOI

dx.doi.org/10.17504/protocols.io.7pnhmme

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DOI: dx.doi.org/10.17504/protocols.io.7pnhmme

Protocol Citation: NUS iGEM 2019. Preparation of Chemically Competent Cells. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.7pnhmme>

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Protocol status: Working



We use this protocol and it's working

Created: September 26, 2019

Last Modified: September 12, 2023

Protocol Integer ID: 28110



1 Transfer  1 mL of overnight culture into  50 mL LB in a flask


Protocol



NAME


Preparation of LB Media

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NUS iGEM**PREVIEW**

1.1 Weigh  25 g of Luria Broth Base powder.



Luria Broth Base (Miller's LB Broth Base)™, powder **Thermo**
Fisher Catalog #12795027


1.2 Add the powder into  1 L of water.




Water refers to sterilized deionized water

1.3 Autoclave entire bottle of LB media.

2 Incubate at  37 °C at  225 rpm until OD600 = 0.6

3 Transfer culture to  50 mL falcon tube

4 Incubate culture on ice for  00:10:00



5 Centrifuge tube at 4 °C , 5000 rpm for 00:05:00

6 Discard supernatant and resuspend pellet in 30 mL of 0.1 Molarity (M) magnesium chloride solution

Protocol



NAME

Preparation of Chemicals

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PREVIEW

6.1 Weigh x grams of desired chemical

6.2 Dissolve in sterile deionized water for IPTG and arabinose or DMSO for ATC

6.3 Syringe filter chemical solution using a 0.22- μ m filter

7 Centrifuge tube at 4 °C , 5000 rpm for 00:05:00

8 Discard supernatant and resuspend pellet in 20 mL of 0.1 Molarity (M) calcium chloride solution



Protocol



NAME

Preparation of Chemicals

CREATED BY

NUS iGEM

PREVIEW

- 8.1 Weigh x grams of desired chemical
- 8.2 Dissolve in sterile deionized water for IPTG and arabinose or DMSO for ATC
- 8.3 Syringe filter chemical solution using a 0.22- μ m filter
- 9 Incubate sample on ice for 00:30:00
- 10 Centrifuge tube at 4 °C , 5000 rpm for 00:05:00
- 11 Resuspend pellet in 1.5 mL of cold mixture comprising 20% glycerol and 80% 0.1 Molarity (M) calcium chloride solution
- 12 Aliquot 60 μ L of mixture into 1.5mL eppendorf tubes
- 13 Store competent cells in -80 °C