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Preparation of Chemically Competent Cells

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Works for me

[dx.doi.org/10.17504/protocols.io.7pnhmme](https://doi.org/10.17504/protocols.io.7pnhmme)

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- 1 Transfer 1 ml of overnight culture into 50 ml LB in a flask



Preparation of LB Media

by NUS iGEM,

National University of Singapore

PREVIEW

RUN

- 1.1 Weigh 25 g of Luria Broth Base powder.



Luria Broth Base (Miller's LB Broth Base)&trade;; powder

by 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 **[M]0.1 Molarity (M)** magnesium chloride solution







Preparation of Chemicals
by NUS iGEM,
National University of Singapore









- 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-µm filter


- 7 Centrifuge tube at  **4 °C** ,  **5000 rpm** for  **00:05:00**
- 8 Discard supernatant and resuspend pellet in  **20 ml** of **[M]0.1 Molarity (M)** calcium chloride solution



Preparation of Chemicals
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- 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% [M]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



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