

Sep 25, 2019

Preparation of Chemically Competent Cells

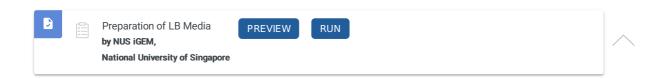
NUS iGEM1

¹National University of Singapore

1 Works for me dx.doi.org/10.17504/protocols.io.7pnhmme



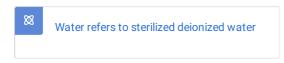
1 Transfer □1 ml of overnight culture into □50 ml LB in a flask



1.1 Weigh **□25** g of Luria Broth Base powder.



1.2 Add the powder into 11 L of water.



- 1.3 Autoclave entire bottle of LB media.
 - 2 Incubate at § 37 °C at <a>325 rpm until OD600 = 0.6

Transfer culture to 50 ml falcon tube Incubate culture on ice for (> 00:10:00 Centrifuge tube at $~~10^{\circ}~4~^{\circ}~C~$, ~~00:05:00~Discard supernatant and resuspend pellet in 30 ml of [MIO.1 Molarity (M) magnesium chloride solution Preparation of Chemicals PREVIEW RUN by NUS iGEM, **National University of Singapore** Weigh x grams of desired chemical Dissolve in sterile deionized water for IPTG and arabinose or DMSO for ATC 6.2 Syringe filter chemical solution using a 0.22-µm filter 6.3 Discard supernatant and resuspend pellet in 20 ml of [M]0.1 Molarity (M) calcium chloride solution Preparation of Chemicals **PREVIEW** RUN by NUS iGEM, **National University of Singapore** Weigh x grams of desired chemical 8.1

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

- 8.3 Syringe filter chemical solution using a 0.22-µm filter
 - 9 Incubate sample on ice for **© 00:30:00**
- 10 Centrifuge tube at $\$ 4 °C , $\$ 5000 rpm for $\$ 00:05:00
- Resuspend pellet in 1.5 ml of cold mixture comprising 20% glycerol and 80% M0.1 Molarity (M) calcium chloride solution
- 12 Aliquot 160 µl of mixture into 1.5mL eppendorf tubes
- 13 Store competent cells in 8 -80 °C

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