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🌐 Preparation of Chemically Competent Cells

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Protocol status: Working

We use this protocol and it's working

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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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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 **Contributed by users**

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-µm 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

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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 µL of mixture into 1.5mL eppendorf tubes

13 Store competent cells in -80 °C