Making electro-competent cells

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Sep 02, 2021

In Development

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dx.doi.org/10.17504/protocols.io.bxxhppj6

iGEM IISER Pune India 2021

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ABSTRACT

This protocol can be used for obtaining of electro-competent bacterial cells.

DOI

dx.doi.org/10.17504/protocols.io.bxxhppj6

PROTOCOL CITATION

Ashwinuday 2021. Making electro-competent cells . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bxxhppj6

CREATED

Sep 02, 2021

LAST MODIFIED

Sep 02, 2021

PROTOCOL INTEGER ID

52937

1 Streak the cells on LB agar plates and incubate at **37 °C** for **12:00:00** 12h

2 Pick single colony and inoculate it in **20 mL** 2X LB broth and incubate at **37 °C** for **12:00:00** - **16:00:00** on shaker incubator 1d 4h

3 Take 2ml of primary inoculum and inoculate it in **200 mL** 2X LB broth and incubate it at **37 °C** on shaker.

3.1 Grow till the OD reaches 0.6

4 Place the culture at **4 °C** for **01:30:00** 1h 30m

5 Aliquot in **50 mL** falcon and spin at **4000 rpm, 4°C, 00:25:00** 25m

6 Discard the supernatant and resuspend well in **40 mL** 10% glycerol

7 Spin at **4000 rpm, 4°C, 00:25:00** 25m

8 Discard the supernatant and resuspend in **25 mL** 10% glycerol

9 Spin at **4000 rpm, 4°C, 00:25:00** 25m

10 Discard the supernatant and resuspend in **20 mL** 10% glycerol. Mix the contents of 2 falcons together such that only 2 falcons of **40 mL** contents is present

11 Spin at **4000 rpm, 4°C, 00:25:00** 25m

12 Discard the supernatant and resuspend in **1 mL** 10% glycerol.

13 Make aliquots of **50 µl** in autoclaved **600 µl** eppendorfs,flash freeze and store at **-80 °C**

Citation: Ashwinuday (09/02/2021). Making electro-competent cells . https://dx.doi.org/10.17504/protocols.io.bxxhppj6