Preparing E. coli cryo cultures

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Abstract

This protocol describes how to prepare bacteria for further storage at -80 °C.

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Guidelines

Always work under sterile conditions to avoid contamination.

Materials

- Glycerol G5516 by Contributed by users

Protocol

Prepare sterile glycerol

Step 1.

Aliquot 99% glycerol and autoclave.

You can also used filtered sterile glycerol.

REAGENTS
- Glycerol G5516 by Contributed by users

Inoculate media

Step 2.

Inoculate 3 mL of your desired media containing appropriate antibiotics with your desired *Escherichia coli* strain. Work under sterile conditions to avoid contamination.

AMOUNT
3 ml Additional info: Media (i.e. LB)

Incubate cultures

Step 3.

Incubate your desired *Escherichia coli* cultures overnight until the culture reaches a exponential to stationary phase and a minimum absorbance of 1.0 and maximum absorbance of 5.0 at OD_{600}.

Centrifuge culture (optional)

Step 4.

Centrifuge your well grown cultures for 10 minutes at 4,000 rpm.

Discard supernatant (optional)

Step 5.

Discard the supernatant quickly. Do not disturb the pellet.

Resuspend (optional)

Step 6.

Resuspend your pellet in 800 µl of your desired media with specific antibiotics.

AMOUNT
800 µl Additional info: media (i.e. LB)

Prepare cryo culture

Step 7.

Pipette 200 µl sterile glycerol in a cryo culture tube. Add 800 µl of your desired resuspended pellet/culture (20 % glycerol (v/v)).

You can also use glycerol concentrations up to 40 % (v/v). Most labs store bacteria in 15-25 % glycerol.

AMOUNT
200 µl Additional info: glycerol
AMOUNT
800 µl Additional info: bacteria culture

Freeze cryo culture

Step 8.

(Alternative: Freeze your cryo cultures with dry ice or liquid nitrogen.)

Store your cryo culture at -80 °C.

Warnings

Always wear appropriate protection equipment while working with liquid nitrogen or dry ice.