

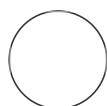


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🌐 General procedure for long-term cryopreservation of Bacteria

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ABSTRACT

A simple and generalized procedure for freezing bacteria cells.

OPEN ACCESS

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protocols.io

<https://protocols.io/view/general-procedure-for-long-term-cryopreservation-o-cpm8vk9w>

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Protocol status: In development
We are still developing and optimizing this protocol

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PROTOCOL integer ID:
77216

50% Glycerol solution

15m

1 Fill a 200 mL Duran bottle with 100 mL PBS



Materials:

⊗ PBS Gibco, ThermoFisher Catalog #20012019

2 Transfer  100 mL 100% Glycerol to Duran bottle

Materials:

 Glycerol **MP Biomedicals Catalog #194680**

3 Sterilize by autoclave at  121 °C for  00:15:00



15m



4 Incubate a  20 mL batch of bacteria overnight

Note

It is important that the bacteria culture is saturated, but not oversaturated. An OD₆₀₀ between 0.4-0.6 is ideal.



5 Aliquot saturated culture into an appropriate container. For example:



 60 µL saturated culture in a  200 µL reaction tube

 600 µL saturated culture in a  1.5 mL reaction tube

 900 µL saturated culture in a  2.0 mL cryotube

6 Mix in 50% glycerol solution. For example:

 40 µL saturated culture in a  200 µL reaction tube

 400 µL saturated culture in a  1.5 mL reaction tube

 600 µL saturated culture in a  2.0 mL cryotube

7 Transfer to an ultra-low temperature or vapor-phase nitrogen freezer for long-term storage

Note

If possible, snap-freeze tubes in liquid nitrogen.