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Bacterial Glycerol Stocks

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the basic steps for the preparation of bacterial stocks in glycerol suitable for long-term storage at -20 or -80.



Preparation of o/n bacterial culture

- 1
 - Prepare liquid culture according to requirements of organism e.g., BHI, TS or LB broth
 - Determine required volume (N vials x 500 µL of culture)
 - Pick several colonies from a purity plate using a sterile loop and inoculate broth
 - Incubate at 37°C o/n if >50mL or 6 h if ~20 mL

Preparation of 50% Glycerol

- 2
 - Measure 10 mL autoclaved glycerol and 10 mL sterile dH₂O into falcon tube
 - Vortex thoroughly to mix
 - Aspirate into 20 mL syringe and pass through a 0.2 µM filter into a sterile falcon tube

Aliquoting and Storage

- 3
 - Add 500 µL glycerol to required number of cryovials
 - Add 500 µL of bacterial liquid culture
 - Vortex briefly to mix
 - Label with ID, preparation date and number (if >1 stock is made)
 - Stocks can be stored at -20°C for up to six months or several years at -80°C