

Feb 08, 2026

Preparation and Handling of Anaerobic Bacteria for Mouse Oral Gavage

DOI

<https://dx.doi.org/10.17504/protocols.io.81wgboqw1lpk/v1>

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Faecalibacterium prausn...



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Protocol Citation: Anastasiya Moiseyenko 2026. Preparation and Handling of Anaerobic Bacteria for Mouse Oral Gavage. protocols.io <https://dx.doi.org/10.17504/protocols.io.81wgboqw1lpk/v1>

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Protocol status: Working

We use this protocol and it's working

Created: February 08, 2026

Last Modified: February 08, 2026

Protocol Integer ID: 242881

Keywords: oral administration, gavage, mouse, anaerobic, bacteria, culture, anaerobic chamber, gaspack, anaeropack, handling of anaerobic bacteria, bacterial culture from an anaerobic chamber, oral administration of bacteria, anaerobic bacteria, transfer of anaerobic, mouse oral gavage, bacterial culture, mouse oral gavage this protocol, preparation

Funders Acknowledgements:

Aligning Science Across Parkinson's

Grant ID: ASAP-020495

Aligning Science Across Parkinson's

Grant ID: ASAP-000375

Heritage Medical Research Institute

Grant ID: n/a

Abstract

This protocol outlines the set up and transfer of anaerobic bacterial culture from an anaerobic chamber for the oral administration of bacteria to animals.

Materials

anaerobic chamber

cryotubes

parafilm

grow flasks

1ml slip-tip syringes

plastic gavage tubes

GasPack box (Anaerobe systems)

AnaeroPack - Anaero sachets (MCG)

pre-reduced 50% glycerol

pre-reduced PBS with 1.5% NaHCO₃

Bacterial growth and aliquots

- 1 Grow anaerobic bacteria inside an anaerobic chamber (80% nitrogen, 10% carbon dioxide, and 10% hydrogen) at 37°C to an approximate optical density (600nm) of 1
- 1.1 For extremely oxygen sensitive bacteria, wrap growth flasks with parafilm prior to removing from anaerobic chamber
- 2 Centrifuge culture and resuspend in sterile pre-reduced 50% glycerol to an approximate Colony-Forming Unit (CFU) density of 10^8 /mL inside the chamber
- 3 Aliquot into cryotubes
- 3.1 For extremely oxygen sensitive bacteria, wrap cryotubes with parafilm prior to removing from anaerobic chamber
- 4 Store aliquots at -20°C for short-term storage, -80°C for long-term storage

Preparation for gavage

- 5 Thaw desired aliquots while preparing gavage supplies
- 5.1 Attach gavage needles to syringes (inside a hood if sterility is of concern) and place inside GasPack box
- 5.2 Place an AnaeroPack - Anaero sachet inside GasPack box, **DO NOT OPEN the sachet until inside the anaerobic chamber**
- 6 Transfer supplies and aliquots into anaerobic chamber
- 7 Transfer aliquots to conical tubes and centrifuge to remove glycerol
- 8 Resuspend culture in pre-reduced PBS with 1.5% NaHCO_3



- 9 Draw up culture into gavage syringes
- 10 Place ready gavage syringes and **opened** AnaeroPack-Anaero sachet in GasPack box, seal tightly
- 11 Remove from chamber and transport to animal facility
- 11.1 For extremely oxygen sensitive bacteria, open the GasPack box as minimally as possible during gavage process and administer gavage as quickly as safely possible to animals to reduce oxygen exposure times