**SOP# 02.101.01**

**Donor fecal preparation – manual homogenization**

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**Purpose**

This SOP describes the procedure used to prepare a fecal homogenate from a fresh fecal sample from a healthy donor.

**Scope**

For exploratory purposes.

**Regulatory References**

NA

**Responsibility**

* Responsibility of experimentalist – understanding and performing this procedure as described; reporting any deviations or problems to area supervisor; adequately documenting the procedures and results
* Area manager or supervisor – ensuring that the analyst performing this procedure is qualified; ensuring that the procedure is followed and update the procedure as necessary

**Definitions/Abbreviations**

* DI water - Deionized water
* L – liter
* mL – milliliter
* uL – microliter
* um – micrometer
* g - gram
* PBS – phosphate buffered saline solution

**Related Documents**

SOP# 03.003.01 – Biosafety cabinet operation and maintenance

SOP# 03.001.01 – Anaerobic chamber operation and maintenance

SOP# 02.040.01 – 50% glycerol buffer preparation

SOP# 03.101.01 – Liquid biological waste disposal

**Required Equipment and Materials / Reagents**

* Class II Type A2 Biosafety cabinet (Labconco), any manufactured biosafety cabinet may be used as long as it is Class II or higher
* Disposable spatula sterilized via autoclaving, any autoclavable disposable spatula may be used. For example VWR catalog # 80081-190
* Sterile, filtered pipettor tips, any brand
* Pipetteman, for example VWR 89079
* Sterile pure grade PBS, for example Amresco catalog# K812
* 50mL tissue grinder (VWR catalog # 47732-450)
* 2-piece specimen collection kit (includes tub and lid, Medline, catalog # DYND36500)
* Cryogenic vials (VWR catalog# 89094-802)
* Bench top scale. Any benchtop scale capable of weighing 1g may be used, for example Mettler Toledo AB54-5
* Sterile 50% glycerol solution (SOP# 02.040.01 – 50% glycerol buffer preparation)

**Precautions**

* Personal protection equipment including gloves, lab glasses, and lab coat must be worn when executing this procedure
* All handling of human fecal matter must be done within a BL2 area inside of a Class II biosafety cabinet
* All work surfaces must be treated with 20% bleach for twenty minutes before and after procedure. 20% bleach mixture must be no more than 7 days old. For the treatment of solid surfaces Wescodyne, Cidex OPA, or Sporicidin maybe be used as alternative disinfectants.

**Procedure**

1. All buffers, tubes, and pipettes will be stored within the anaerobic chamber for no less than 12 hours before sample processing
2. Donor will bring fresh fecal material, in 2-piece collection kit, to the BL2 lab space within 40 minutes of passage. Time of sample arrival and time since passage will be noted in the experimentalist’s lab notebook.
3. Bring sample into anaerobic chamber (SOP# 03.001.01 – Anaerobic chamber operation and maintenance).
4. All of the following takes place within the anaerobic chamber unless otherwise noted
5. Aliquot 10mL of glycerol solution into 50mL tissue disrupter tube.
6. Use sterile disposable spatula to place 1g of fecal matter into tissue disrupter tube using benchtop scale.
7. Dispose of used spatula in biological waste bin.
8. Bring disruptor tube into anaerobic chamber.
9. Manually homogenize sample into buffer by hand grinding the disrupter pestle for one minute
10. Let tube sit for 10 minutes so large particulates can settle to the bottom of the tube
11. Aliquot 200uLs of supernatant and 200uLs of 50% glycerol solution into at least three cryogenic vials for future use. Store these at -80°C.
12. The remaining material can be used for bacterial isolation (see SOPs #02.201.01 - 204.204.01) or disposed of as liquid biological waste (SOP# 03.101.01 – Liquid biological waste disposal).

**Version History**

This is the first version of this document.

**Worksheets**

NA

**Appendix**

NA