**SOP# 02.102.01**

**Donor fecal preparation - Whirlpak**

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**Purpose**

This SOP describes the procedure used to collect an aliquot from a full fecal sample provided by a healthy donor

**Scope**

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 (see SOP# 02.040.01 – 50% glycerol buffer preparation)
* Whirl-pak 3mm filter bag
* EasyMix bench top homogenizer
* Small autoclave bags, used for secondary containment, any brand

**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

**Procedure**

1. All buffers, tubes, and pipettes will be stored within the anaerobic chamber for no less than 12 hours before sample processing (SOP# 03.001.01 – Anaerobic chamber operation and maintenance).
2. Donor will bring fresh fecal material, in 2-piece collection kit, to the BL2 lab space within 40 minutes of passage.
3. Weight collection tub using bench top scale (use empty tub to tare scale)
4. Calculate volume of buffer required for sample, 1g feces /10mL buffer
5. Bring sample into anaerobic chamber (SOP# 03.001.01 – Anaerobic chamber operation and maintenance).
6. All of the following takes place within the anaerobic chamber unless otherwise noted
7. Use sterile disposable spatula to place as much of the fecal sample as possible into one side of the Whirlpak filter bag
8. Dispose of used spatula in biological waste bin
9. Add required amount of glycerol solution to same side of Whirlpak bag that the sample is in
10. Carefully roll and close Whirlpak bag to contain sample, carefully press out as much air as possible while doing so
11. Place Whirlpak bag into a secondary containment bag.
12. Place secondarily contained Whirlpak bag into EasyMix and homogenize for 60 seconds
13. Remove Whirlpak bag form EasyMix and remove secondary containment bag
14. Filtered and homogenized sample will be located on the opposite side of the filter (Relative to side that you loaded the sample on to)
15. Aliquot 200uLs of filtered/homogenized material and 200uLs of 50% glycerol solution into at least three cryogenic vials for future use
16. The remaining material can be used for culturing of isolates (see SOPs #02.201.01 - 204.204.01) or disposed of as liquid biological waste (SOP# 03.101.01 – Liquid biological waste disposal).
17. Dispose of used Whirlpak bag in solid biological waste bin.

**Version History**

This is the first version of this document

**Worksheets**

NA

**Appendix**

NA