**SOP# 02.040.01**

**Glycerol Buffer Preparation**

**Author: Allison Perrotta**

**Date of Rev.: September 8, 2016**

**Page 1 of 3**

**Purpose**

This SOP describes the procedure used to prepare 1 L of a 50% glycerol solution used in making freezer stocks of bacterial isolates.

**Scope**

For exploratory and isolate maintenance 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 updating the procedure as necessary

**Definitions/Abbreviations**

uLs – microliters

mLs – milliliters

g - grams

**Related Documents**

SOP# 02.080.01 – Pre-reduced media and buffers - liquid sparging

**Required Equipment and Materials / Reagents**

L-cysteine (Sigma Aldrich, C7532)

Bottle-top vacuum filter system pore size 0.2 μm- 1L (Sigma, Cat# CLS430515)

MilliQ Water

Glycerol, any glycerol of 99+% purity may be used for example CAS# 56-81-5

1L clean, glass bottle and lid

500mL clean graduated cylinder

Benchtop scale, any benchtop scale capable of weighing 1g of material may be used for example Mettler Toledo AB54-5

Benchtop stir plate, any magnetic stir plate may be used, for example VWR Catalog# 10153-308

Clean stir bars, any magnetic stir bar my be used, for example VWR Catalog# 85948-116

**Precautions**

Personal protection equipment including gloves, lab glasses, and lab coat must be worn when executing this procedure

**Procedure**

1. Use the graduated cylinder to measure out 500mLs of glycerol and pour this into the 1L glass bottle
2. Without previously rinsing, use the same graduated cylinder to portion out 500mLs of milliQ water and pour into the same 1L glass bottle
3. Weigh out 1g of L-cysteine and add to the 1L glass bottle
4. Add a stir bar to the 1L glass bottle and allow to stir on the stir plate until all of the l-cysteine is dissolved
5. Connect the vacuum filtration system up to the in-house vacuum line

Note: A stand alone vacuum pump may be used if no in-house vacuum line is available

1. Label the bottom bottle of the filtration system with “50% glycerol, 0.1% cysteine” and the date and experimentalist’s name
2. Pour the mixed glycerol solution into the top of the filtration system
3. Turn on the vacuum
4. When all of the buffer has moved from the top to the bottom of the filtration system turn off the vacuum
5. Carefully unscrew the top of the filter system and cap the bottom bottle (now containing the buffer) with the sterile lid provided with the filtration system.
6. Buffer can now be kept at the bench and sparged for use as needed (SOP# 02.080.01 – Pre-reduced media and buffers - liquid sparging), or can be brought into the anaerobic chamber and allowed to reduce over night.

**Version History**

Version 1 of 1

**Worksheets**

NA

**Appendix**

NA