**SOP# 02.291.01**

**Bacterial frozen stock maintenance**

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**Date of Rev.:**

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**Purpose**

This SOP describes the procedure used to maintain a supply of uncontaminated isolate stocks, through the generation of fresh freezer stocks from single bacterial colonies. This procedure should be performed each time an experimentalist utilizes a bacterial stock.

**Scope**

For quality control 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**

* Culture – an actively growing cultivation of an isolate in liquid media
* Revived freezer stock – A glycerol stock made from an aliquot of a culture, used as the stock of each particular isolate after revival to limit freeze/thaw and cover opening cycles on the primary isolation plate.

**Related Documents**

SOP# 03.001.01 – Anaerobic chamber operation and maintenance

SOP# 03.003.01 – Biosafety cabinet operation and maintenance

SOP# 02.040.01 – 50% glycerol buffer preparation

SOP# 02.041.01 – 20% bleach solution preparation.

SOP# 02.281.01 - Bacterial Isolate Revival

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

**Required Equipment and Materials / Reagents**

* + Previously revived freezer stock (e.g. a stock generated using SOP# 02.81.01 – Bacterial Isolate Revival)
  + Class II Type A2 Biosafety cabinet (Labconco), any manufactured biosafety cabinet may be used as long as it is Class II or higher
  + 50% glycerol buffer (SOP# 02.040.01 – 50% glycerol buffer preparation)
  + 20% bleach solution (SOP# 02.041.01 – Bleach solution preparation)
  + Cryogenic vials (VWR catalog# 89094-802)
  + Freezer boxes, for example Argos catalog# R3027A
  + -80°C freezer
  + Freezer racks, for example VWR catalog# 89128-116

**Precautions**

* Personal protection equipment including gloves, lab glasses, and lab coat must be worn when executing this procedure
* All handling of human-derived isolates must be done within a BL2 area inside of a Class II biosafety cabinet or the anaerobic chamber
* All work surfaces must be treated with 20% bleach (volume/volume) twenty minutes of contact time before use, and 20 minutes after use. 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. Remove cryogenic vial with desired isolate from -80°C freezer
2. Bring vial into the anaerobic chamber or biosafety cabinet as needed. See SOP# 03.003.01 - Biosafety cabinet operation and maintenance and SOP# 003.001.01 – Anaerobic chamber operation and maintenance.

Note: Steps 3 – 12 of this procedure must be performed in the anaerobic chamber.

1. Carefully open the vial.

Note: this should be done while volume in vial is still frozen to avoid splashing. Dry ice may be used while carrying vial to work area to ensure it remains frozen

1. Use inoculation loop to sample isolate stock, and streak out sample onto agar plates of desired media.
2. Close vial tightly and return to -80°C freezer.
3. Using a permanent marker, mark cap of vial with a dot to indicate that it has been opened. Note date that the was originally stock was created and isolate name in lab notebook.
4. Place streaked agar plates into the 37°C incubator
5. Check growth of plates after 24 hours
6. If single distinct colonies have grown continue to step 11, otherwise wait another 24 hours or until single distinct colonies have grown

Use an inoculation loop to pick a single colony from the plate and inoculate 7mLs of desired reduced liquid media in a culture tube (SOP# 02.080.01 - Pre-reduced media and buffers - liquid sparging)

1. Once the culture is turbid, generally 24 - 48 hours later, gently vortex the culture for one second.
2. Combine 200uLs of culture and 200uLs of 50% glycerol buffer in a new labeled cryogenic vial.

Note: The label must include isolate identity, ,information and date

1. The remaining volume of the culture may be used for other procedures
2. Remove the vial form the anaerobic chamber (SOP# 003.001.01 – Anaerobic chamber operation and maintenance)
3. Store the new isolate freezer stock in a freezer box within the -80°C freezer
4. Always use a new, unopened freezer stock for future use. Do not return to previously opened stocks, as they may be contaminated. However, opened stocks should be stored for at least 3 years after use for documentation purposes.

**Version History**

NA (This is the first version of this SOP.)

**Worksheets**

NA

**Appendix**

NA