

SOP-M012

Glycerol Samples (Bacteria) for -80° Freezer

Objective: To store bacteria using 20% glycerol + Broth in the -80° freezer

Products:

Cryule Vial Polypropylene Sterile: Wheaton Cat#982745 (50pcs)

Disposable inoculating Loop. (VWR cat#90001-100 BD Difco Blue 10ul)

Glycerol: 20% Solution

Broth: You can use either TSA or NZE, follow directions on the container.

Procedure to prepare 20% Glycerol + TSB solution:

1. To make the 20% Glycerol + TSB solution, first make up the TS Broth by dissolving 12 grams of BBL Trypticase Soy Broth in 400 mL of Millipore water.
2. Use stir bar to mix the powder into the water completely while also warming the solution on a hotplate.
3. Once the powder has completely dissolved in the water, transfer the solution to a pre-autoclaved 500 mL bottle and then autoclave solution at 121°C for 15 minutes.
4. Be sure to test the solution for any contamination after autoclaving by plating 50 µL of the TSB and incubating for 24 hours at 37°C.
5. Confirm that the plates have no bacteria or microorganism growth and then continue to prepare the solution.
6. For a 500 mL of 20% Glycerol + TSB solution, add 100 mL of Glycerol to 400 mL of TSB using sterile technique.
7. Mix the solution by swirling the bottle and place in the cold room for later use.

Procedure to store bacteria in Cryule vials:

1. Make a new streak plate of the bacteria you want to check for viability from recent or older plates or slants. If you get growth from the streak, take a few colonies and do a serial dilution to grow single colonies.
2. Spray a tray with 70% ethanol and place in the sterile hood. In that tray, put as many Cryule vials as you need for each bacteria. Label each vial with the bacteria PU number, type and date.
3. In each Cryule vial, put 1.5mls of the Broth+20% glycerol solution.
4. From your original streak of the bacteria, use the inoculating loop and take 3 to 4 colonies. You want the concentration to be high.
5. Using the loop, rub it on the side of the Cryule vial to remove most of the colonies. Then with a swirling motion, remove the remaining colonies from the loop. Be sure it is well blended in the glycerol mixture. If not, vortex until blended.
6. Repeat steps 2 through 5 for each of the different bacteria samples you want to store in the -80° freezer.

7. Place the Cryule vials in a freezer box and label the outside with the necessary information. Plate this box in the -80° freezer for long term storage.

Created by: Cheryl Holdman & Rachael Eberle & Sabeen Nadir

Verified by: _____ **Date:** 2/27/2014

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