**Tryptic Soy Broth w/ 20% Glycerol Production SOP**

**Purpose**

While pre-aliquot tryptic soy broth (TSB) w/ 20% glycerol is commercially available, costs associated with these materials is volatile and can be limiting to research budgets. This alternative can be utilized for bio-banking in situations where the costs of pre-formulated and aliquot versions are not viable options.

**Materials & Equipment**

* Teknova Tryptic soy broth; IL, Fisher Catalog No. 50-843-291 *or* equivalent
* Sigma-Aldrich Glycerol (99.5%); 100mL, Sigma Catalog No. G9012-100ml *or* equivalent
* 15/50mL conical vials

**Protocol**

**Note**: Batches of TSB/20% glycerol are made by the liter, which are then aliquotted into smaller working volumes. This limits the potential for contamination.

1. Obtain a 1-liter bottle of TSB. Using aseptic technique, decant 200mL from the bottle into a sterile vessel. Mark the vessel with the identity of the reagent, the date aliquotted and an expiration date. **Note**: these aliquots of TSB can be saved until 800mL can be combined to create another batch of TSB/20% glycerol.
2. Add 200mL of glycerol to the 1L bottle containing 800mL of TSB. **Note**: Glycerol is very viscous. If using 100mL vials of glycerol, you can dump one at a time into the TSB and leave them upside down in the mouth of the liter bottle for a few minutes to make sure all the glycerol has been removed.
3. Tightly cap the liter bottle and gently rotate to homogenize the glycerol within the TSB. Ensure that no un-homogenized glycerol remains at the bottom of the bottle.
4. Label the 1-liter bottle “TSB w/ 20% glycerol,” along with the date created and the study for which it was created for.
5. Aliquot the TSB w/ 20% glycerol into a smaller working volume of 15 or 50mL and label these volumes with the identity of the material, the date aliquotted, and an expiration date. The entire liter need not be aliquotted all at once; the remainders of the liter can be stored in the primary container at your bench.

**References**

None