Freezing E. coli

Pink colonies = positive for O157:H7

1 positive colony in 5 ml TSB (Tryptic Soy Broth)

Let incubate overnight

centrifuge at 400rmp for 7mins

Discard supernant

Pellet,

700 microliters of TSB and

300 microliters of Glycerol

store in freezer vial store at -80 degrees celcius

Recipe for TSB

**Tryptic Soy Broth (TSB)**

This is a general purpose broth medium prepared for the cultivation of fastidious and non fastidious organisms.

**Materials for preparing 1 L of TSB**

* 30 g dehydrated TSB medium
* 1 L distilled or deionized water
* Sterile 20 mL glass bottles with lids.

***Method***

1. Dissolve media in 1 L of water.

2. Warm slightly to dissolve completely.

3. Dispense 9 mL aliquots into the glass bottles.

4. Sterilize in the autoclave at 121°C for 15 minutes

1x TSB Plates

Recipe for 1L:

30 g of TSB powder

15 g of bactoagar

950 mL Water (or fill bottle up to 1 L mark)