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Working

Ultra-freeze media

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ABSTRACT

This is a recipe for an "ultra-freeze" meda recipe. Taken stock from Gibson *et al.* This recipe enables the quicker recovery of cells stored as a glycerol stock at -80. Cells revived when frozen in this medium seem to revive quicker, especially when inoculated into minimal media.

A bit onerous to prepare when compared to the standard 15%v/V glyceorl stock approach but can cut up to 24hrs in revival time, very valuable when dealing with slow growing undomesticated microorganisms.

GUIDELINES

Final product has quite a big of heterogeneous protein matter in it that will settle and stick together over time. Recommened include a magnetic stirbar to ease sterile stirring of prepared stock solution.
Standard 30 min liquids cycle is OK in preparation.

MATERIALS

NAME	CATALOG #	VENDOR
Glucose		
Glycerol	GB0232.SIZE.500ml	Bio Basic Inc.
Tryptone Soya Broth	CM0129	Oxoid Microbiology Products - Thermo Fischer
Skim Milk Powder	View	

100mL recipe

1 Mix Together the following reagents

- **3 g** Tryptone Soya broth
- **0.5 g** Glucose
- **2 g** Skim milk powder
- **4 g** Glycerol

2 Autoclave for 30 minutes

3 Solution should be turbid, heterogeneous. Store at RT

Using Ultra-freeze media

4 The paper suggests growing up a lawn but I have found 5mL of liquid culture at stationary phase of chosen microbe works just as well. Just need lots of stationary phase cell material to resuspend in ultra-freeze media for storage.

Inoculate overnight culture in media volume that will yield about similar wet cell mass of 5mL of *Escherichia coli*.

5 If using a lawn of microbes... scrape clean plate of solid media containing lawn of microbe monoculture. Resuspend goop in 1mL of ultrafreeze media, preferabbly in a labeled cryovial.

If using stationary phaes culture. Pellet cells, spin down at 5000rcf for 10 minutes. Pipette off supernatant and resuspend pellett in 1mL of ultrafreeze media, preferrably in a labeled cryovial.

6 Freeze!
Store cryovial with ultra-freeze resuspension in -80.

Recovery

7 Scrape a little bit of ice from cryobial. Inoculate onto solid or liquid media and wait for growth. In my experience the pellett is pretty hard. It may be worthwhile to modify the recipe to 18.75g for more vitrious ice. However, 4g glyceorl in a 100mL solution, does not seem to be particularly pernicious to the microbes.