

Methanotroph glycerol stock preparation

1. Inoculate 30 mL of fresh NMS with a loopful of methanotroph from plate or 3 mL from starter liquid cultures.*
2. Incubate culture at appropriate temperature, 220 rpm until late exponential or early lag phase.**
3. Transfer culture to disposable 50-mL polypropylene tubes.
4. Centrifuge culture at $4,300 \times g$ for 10 min.
5. Decant spent medium from the cell pellet, then resuspend pellet in 0.1 V of fresh NMS (3 mL).
6. In a sterile 2-mL screw-cap cryotube or microcentrifuge tube, add 0.7 mL of suspended cell and 0.3 mL of sterile 50% glycerol solution. ***
7. Invert tube 5 – 6 times or until the stock solution is well mixed and does not have any layers.
8. Label directly on both the cap and tube, then store in a cryobox at -80°C .

* Appropriate starting OD = 0.05 ~ 0.1

** Typically 2 – 3 days and OD = 0.3 ~ 0.6, depending on methanotroph strain

*** 15% glycerol stock

***E. coli* glycerol stock preparation**

1. Pick a single colony from a plate of *E. coli* and inoculate 3 mL of LB medium in 15-mL sterile tube. Incubate the culture overnight at 37°C , 220 rpm.*
2. In a sterile 2-mL screw-cap cryotube or microcentrifuge tube, add 0.7 mL of culture and 0.3 mL of sterile 50% glycerol solution.
3. Invert tube 5 – 6 times or until the stock solution is well mixed and does not have any layers.
4. Label directly on both the cap and tube, then store in a cryobox at -80°C .

* The culture can be scaled up to 10 mL as long as the culture has 4 V headspace for sufficient aeration.