Mm Control Media (Green Cap)

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1 Works for me
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Equipment for making media

1 For making media you will need:
2 --> 500 mL graduated cylinders
1 --> 100 mL graduated cylinder
1 --> 10 mL graduated cylinder
2 --> small funnels
2 --> weigh boats
1 --> lab spatula
1 --> pipette + tip
1 --> 1000 mL or 2000 mL flask (depending on if you're making 1 L or 2 L of media)

Making Media (Before autoclave) for 1 L

2 Pour 500 mL of nanopure water into beaker

3 Add 2.28 g of NaCl

4 Add 100 mL of 10x general salts sol'n

5 Add 0.82 g of sodium acetate, anhydrous

6 Add 5.6 mL of 60% Na DL-lactate syrup

6.1 IF MAKING LACTATE-FREE MM MEDIA, DO NOT ADD THE SYRUP.

7 Add 1 mL of resazurin

8 The remaining 375 mL are nanopure water

9 **For 2 L of media, you will add**:
- 1,000 mL of nanopure water
- 4.6 gof NaCl
- 200 mL of 10x general salts
- 1.64 g of sodium acetate, anhydrous
- 11.2 mL of Na DL-Lactate Syrup (or skip if Lactate-Free)
- 2 mL of resazurin
- 750 mL of nanopure water

10 ⚠

If not immediately autoclaving, place aluminum foil (and a rubber stopper if wanted) on the top to prevent evaporation, label, and store in fridge.

Making media (autoclaving process and initial hooking up to gas) for 1 + 2 L

11 When autoclaving:
- Pour media that was mixed prior into the Widdel flask
- ensure that the red caps are loosened
- put aluminum foil on both ends of the black rubber tubes
- place autoclave tape of the flask
- select the "P12 30 min liquid" program

12 After autoclaving, connect the Widdel flask to N2CO2:
- Turn on the gas tank
- Add new plastic filter and needle tip on the cannula tip that will be going through the rubber tube with the cotton filter
- Place the needle with the flowing gas into the rubber tube w/ the cotton filter and ensure that gas is running into widdel flask (red caps will rattle or you can check by turning on the air flow to the other cannula tip)

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13 Once connected to N2CO2, bring the media to room temperature by:
- Placing flask into red ice bucket on top of stir plate and putting ice around it + turning on stir plate
- pour a bit of cold water + more ice to speed up the cooling process

14 If you haven't done so beforehand, while the media cools, it is a perfect opportunity to autoclave a sufficient amount of clean balch tubes and stoppers

After media has been hooked up to gas and has cooled

15 Remove ice bucket from below the widdle flask and readjust the apparatus holding it upright.

16 For 1 L of media you will need:
- 1.1 mL of 1 M K2HPO4
- 25 mL of 1 M NaHCO3
- 1 mL of trace minerals
- 1 mL of Thauer's vitamins
- 1 mL of 1 M cysteine
- 1 mL of 7.8% w/v Na2S

17 For 2 L of media you will need:
- 2.2 mL of 1 M K2HPO4
- 50 mL of 1 M NaHCO3
- 2 mL of trace minerals
- 2 mL of Thauer's vitamins
- 2 mL of 1 M cysteine
- 2 mL of 7.8% w/v Na2S

18 Pour 70% ethanol sol'n on top of the containers of the aforementioned ingredients and use a flame to sterilize them

19 Using a new needle and tip each time to maintain sterilization (1 mL, 3 mL, 5 mL, and 30 mL syringes + 23 gauge needles will be used) add the aforementioned chemicals into the waddel flask through the rubber membrane in the red cap

20 Once everything has been added, allow the media to reduce (it will go from blue --> purple --> pink --> clear) before dispensing into tubes

To dispense reduce media into tubes:

21 Sterilize the cunnula that will be dispensing the gas into the balch tubes as well as thin tweezers using the ethanol and flame process

22 Remove the cotton filter from the top of a 5 mL pipette with the sterile tweezers

23 Attach the pipette to the end of the black rubber tube with the dispensing clamp (ensure this is done in a sterile manner)

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24 Ensure that the red caps on the Waddel flasks are tightened and tape down the black stopper to avoid it being popped out from built up pressure

25 Ensure that all clamps are loosened and squeeze the dispensing clamp to test the flow. Ensure that there is a waste bucket below to catch any dripping media.

26 Dispense 12 mL of media into each balch tube (This will be achieved by using the 3.9 mL mark on the 5 mL pippette as a guide)

27 Stopper the tube while pulling out the gassing cannula

28 After all media has been transferred to the tubes, crimp an aluminum seal onto the cap (green)

28.1 For Lactate-Free Mm tubes, make sure to add 2 sharpie lines along the edges of the gren caps to differentiate with other Mm media

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