Media preparation

Version 1.4

Gleb Ebert

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General notes on autoclaving

- Place lid on bottle, screw until it starts gripping and then turn it back a full rotation. Then tape the lid in this position with a piece of autoclaving tape. This is to ensure that expanding gases can escape the bottle and it doesn't explode
- We usually run a short 15/20 minute liquid cycle (number 006), but longer liquid cycles are permissible

Minimal Marine Sea Water (MSW) + D-Gluconic Acid 0.1% (1 liter)

1. Autoclave one empty 1 L bottle and 1 L of MilliQ in a separate bottle
2. After autoclaving, add MilliQ and magnetic stir bar to beaker. Then, add the following chemicals (use high-precision scale in antibiotic station for the last two ingredients):
    - 1.0g D-Gluconic acid sodium salt
    - 1.0g KH_2PO_4
    - 1.0g $H_4N_2O_3$
    - 10.0g NaCl
    - 0.2g $MgSO_4 \cdot 7H_2O$
    - 10mg $FeSO_4 \cdot 7H_2O$
    - 10mg $CaCl_2 \cdot 2H_2O$
3. Adjust pH to 7
    1. Turn on pH meter, take electrode out of storage buffer, rinse with MilliQ water and carefully dry with lab wipes
    2. While stirring, lower the electrode into the liquid medium and press the measure button
    3. Add NaOH slowly until the pH reaches 7 (6.95 - 7.05 are permissible). 1000 mL medium require ~ 1000 uL of NaOH
    4. When done, rinse and dry the electrode before putting in back into the storage solution and turninig off the pH meter
4. Sterile filtration
    1. Inside a sterile hood, screw the sterile filter onto the autoclaved empty bottle
    2. Back in J18, turn on the vacuum pump and stick the hose onto the sterile filter nozzle
    3. Add the liquid medium (and any remaining autoclaved water) to the sterile filter
    4. First take the hose off the nozzle, then turn off the vacuum pump
    5. Finally, screw off the sterile filter in a hood and close the bottle with its cap

5. Clean up and storage

    1. Wipe bench and scales
    2. Turn off stirring plate, scales and pH meter (if not off already)
    3. Put away dry beakers, spatulas and weighing boats before washing your own ones
    4. Label liquid medium bottle with “MSW + D-Gluc.”, your name and the date
    5. Store in the fridge at 4°C

Minimal Marine Sea Water Agar 1% (MSWA) + D-Gluconic Acid 0.1% (1 liter)

1. Dissolve 15g of BactoAgar in 800 mL of MilliQ, then add to 1 L bottle
2. Autoclave agar and 200 mL of MilliQ in a separate (e.g. 250 mL) bottle
3. After autoclaving, add 200 mL MilliQ and magnetic stir bar to beaker. Then, add the following chemicals (use high-precision scale in antibiotic station for the last two ingredients)

    - 1.0g D-Gluconic acid sodium salt
    - 1.0g KH_2PO_4
    - 1.0g $H_4N_2O_3$
    - 10.0g NaCl
    - 0.2g $MgSO_4 \cdot 7H_2O$
    - 10mg $FeSO_4 \cdot 7H_2O$
    - 10mg $CaCl_2 \cdot 2H_2O$

4. Adjust pH to 7

    1. Turn on pH meter, take electrode out of storage buffer, rinse with MilliQ water and carefully dry with lab wipes
    2. While stirring, lower the electrode into the liquid medium and press the measure button
    3. Add NaOH slowly until the pH reaches 7 (6.95 - 7.05 are permissible). 1000 mL medium require ~ 1000 uL of NaOH
    4. When done, rinse and dry the electrode before putting in back into the storage solution and turninig off the pH meter

5. Sterile filtration

    1. Inside a sterile, screw the sterile filter onto the autoclaved bottle containing 800 mL agar
    2. Back in J18, turn on the vacuum pump and stick the hose onto the sterile filter nozzle
    3. Add the liquid medium (and any remaining autoclaved water) to the sterile filter
    4. First take the hose of the nozzle, then turn off the vacuum pump
    5. Finally, screw off the sterile filter in a hood and close the bottle with its cap. Mix well by swirling and inverting

6. Clean up and storage

    1. Wipe bench and scales
    2. Turn off stirring plate, scales and pH meter (if not off already)
    3. Put away dry beakers, spatulas and weighing boats before washing your own ones
    4. Label medium bottle with “MSWA + D-Gluc.”, your name and the date. Preferably pour agar plates immediately (otherwise heat-sensitive components might decay). Otherwise store in media incubator at 50°C

Marine broth (MB) (1 liter)

0. First, check the Marine broth 2216 (Sigma Aldrich) bottle for instructions. If they exist, follow those instead of this protocol.
1. Add 37.4 g of Marine broth 2216 powder to roughly 900 mL of MilliQ. Once dissolved, fill volume up to 1000 mL, then add to 1L bottle
2. Autoclave and store in the fridge at 4°C

Marine agar (MA) (1 liter)

0. First, check the Marine broth 2216 (Sigma Aldrich) bottle for instructions. If they exist, follow those instead of this protocol
1. Add 15 g of BactoAgar to roughly 900mL of MilliQ
2. Add 37.4 g of Marine broth 2216 powder the beaker. Once dissolved (excluding the agar), add to 1 L bottle
3. After autoclaving, store bottle in 50°C incubator to prevent solidifying

Casitone-Tris (CTT) liquid (1 liter)

1. Dissolve the following components in 900 mL MilliQ:
    - 10 mL 1.0 M Trish-HCl (pH 8.0)
    - 10 mL 0.8 M $MgSO_4$
    - 10g Bacto Casitone
2. Fill up volume to 1 L with MilliQ and adjust pH to 7.6
3. Autoclave using cycle 003 (liquid media, 50 min at 121°C)
4. After cooling <50°C add 1 mL 1 M KH_2PO_4-K_2HPO_4

Phosphate-Buffered Saline (PBS; 1 liter of 10x solution)

Instructions according to Sigma

1. Dissolve the following components in 900 mL MilliQ:
    - 17.8 g of $Na_2HPO_4 \cdot 2H_2O$
    - 2.4 g of KH_2PO_4
    - 80 g of NaCl
    - 2 g of KCl
2. Adjust final volume to 1 L with MilliQ
3. Measure pH using the pH meter. The pH of the 10X stock will be approximately 6.8, but when diluted to 1x PBS the pH should change to 7.4
4. Autoclave or filter-sterilize into a sterile bottle
5. Store in the fridge at 4°C