Preparation of Plate-Count Agar (PCA)

1. SCOPE AND APPLICATION

1.1. This method is based on Difco® medium instructions for preparing agar. It is used for long term maintenance of algal cultures.

2. SUMMARY OF METHOD

2.1. Agar is added to appropriate liquid medium, melted, poured into Petri dishes, covered and allowed to cool. Plates are stored in the walk-in refrigerator until needed for culturing algae.

2.2. Interferences:

2.2.1. An aseptic technique must be used to ensure there is no contamination of the culture via the media, reagents, supplies or the analyst.

2.2.2. Use Class-A glassware to ensure accurate volume measurements.

2.2.3. Avoid burning or boil-over of media. Overheating can cause nutrient degradation.

3. EQUIPMENT AND SUPPLIES

3.1. 500 mL Erlenmeyer flask, 2

3.2. 250 mL Graduated cylinder, 2

3.3. Weigh boats, 2

3.4. Mettler Toledo 403TS/00 balance, calibrated per SOP TA-06.34

3.5. Hot-plate with stirrer (Corning® Model 320)

3.6. 1” Magnetic stir-bars, 2

3.7. Autoclave gloves

3.8. 100 x 15 mm Sterile disposable Petri dishes, 20 (Falcon® 1029)

3.9. Labeling tape, 4 pieces

3.10. Permanent black marker

3.11. Clean spatula

3.12. Parafilm®

3.13. Computer with access to the Laboratory Information Management System (LIMS)

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4. REAGENTS AND STANDARDS

4.1. Preliminary Algal Assay Procedure (PAAP) freshwater alga media, prepared in advance per SOP TA-03.03.

4.2. Artificial Seawater (ASW) saltwater alga media, prepared in advance per SOP TA-03.04.

4.3. Bacto-Agar, dehydrated. (Difco® Bacto-Agar stored in the desiccator in the Microbiology Lab.)

5. MEDIA PREPARATION

5.1. Label one 500 mL flask and one 250 mL cylinder “PAAP.” Label the other 500 mL flask and 250 mL cylinder “ASW Media”.

5.2. Separate the Petri dishes into two groups of 10. Label the top of each dish of one set with the batch number and “PAAP Agar.” Label the other set with the batch number and “ASW Media Agar”. On each petri dish, add an expiration date of one year from the preparation date.

5.2.1. The batch number is the date that the solution was made.

5.3. Using the graduated cylinder labeled “PAAP,” measure and pour 250 mL of PAAP media into the 500 mL flask labeled “PAAP.”

5.4. Using the graduated cylinder labeled “ASW media,” measure and pour 250 mL of ASW media into the 500 mL flask labeled “ASW Media”.

5.5. Using the balance, a weigh boat, and the clean spatula, weigh out 3.5 g of Bacto-Agar and add it to the “PAAP” flask.

5.6. Put a magnetic stir-bar into the “PAAP” flask.

5.7. Place the “PAAP” flask on the hot-plate stirrer. Adjust the temperature control to “high.” Adjust the stirrer speed as needed to cause a small vortex to form at the surface of the liquid.

5.8. Monitor the liquid at the bottom of the flask for air bubble formation and monitor the surface of the liquid for the formation of foam. These are indicators that the liquid is about to boil.

5.9. **WARNING-HOT LIQUID!** As soon as the foam starts to rise toward the mouth of the flask, use the autoclave gloves to remove the flask from the hot-plate stirrer. The liquid will rapidly foam out of the flask if it is not removed from the hot plate. Place the flask on the countertop.

5.10. Set the hot-plate stirrer controls to the “off” position.

5.11. Use the autoclave gloves to carry the “PAAP” media flask to the “PAAP Agar” Petri dishes. Remove the top from a “PAAP Agar” Petri dish and carefully pour the “PAAP” liquid into the bottom half of the Petri dish, filling the dish approximately one-half

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full (~25 mL). Place the top back on the Petri dish. Repeat for the remaining nine labeled dishes.

5.12. Repeat Steps 5.5-5.11 using the flask labeled “ASW Media” and Petri dishes labeled “ASW Media Agar”.

5.13. Take both flasks to a sink, use copious amounts of hot water and rinse any remaining agar down the drain.

5.14. Allow the Petri dishes containing the agar to cool for approximately 1 hour or until the agar has solidified.

5.15. After the agar has solidified, wrap with Parafilm®, then invert the dishes (bottom up and top down).

5.16. Put the Petri dishes in the walk-in refrigerator.

5.17. Record batch information in LIMS Standard Prep Tracker.

6. DATA ARCHIVAL

6.1. FDEP Laboratory Information Management System (LIMS)

7. QUALITY CONTROL

7.1. Autoclaved, Class-A glassware is used to minimize the potential for contamination and to ensure accurate volume measurements.

7.2. Two “Class 1” weights that bracket the desired weights should be weighed and recorded before reagents are weighed. See SOP TA-06.34.

7.3. Media is monitored during heating to make sure it does not burn or boil over.

7.4. Petri dishes are stored upside down to prevent condensation pooling on the agar.

7.5. Media is not used after the expiration date.

8. SAFETY/HAZARDOUS WASTE MANAGEMENT

8.1. Proper laboratory protection, including lab hood or ventilation system, lab coat, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

8.2. Expired media is taken to the microbiology lab and placed in the “to be autoclaved” bag for disposal. (Refer to DEP Laboratory Quality Manual for media disposal.)

8.3. Refer to the DEP Laboratory Health and Safety Manual.

8.4. Read MSDS information sheets for reagent handling and disposal.

9. REFERENCES

9.1. DEP Laboratory Quality Manual

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DEP Laboratory Quality Assurance Manual and SOPs | Florida Department of Environmental Protection

9.2. DEP Laboratory Health and Safety Manual https://www.floridadep.net/resources/health-safety-manual

9.3. Difco® medium instructions for preparing agar

9.4. DEP SOP TA-03.03, Preparation of Preliminary Algal Assay Procedure (PAAP) medium for Freshwater Algae Cultures

9.5. DEP SOP TA-03.04, Preparation of Artificial Seawater (ASW) Medium for Culturing *Dunaliella tertiolecta*

9.6. DEP SOP TA-06.34, Calibration and Use of Mettler Toledo 403TS/00 Balance

APPENDIX OF CHANGES

12/11/2007 Added Scope an Application and Summary of Method.

11/07/2008 Added References.

04/30/2015 Formatted for 508 compliance.

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