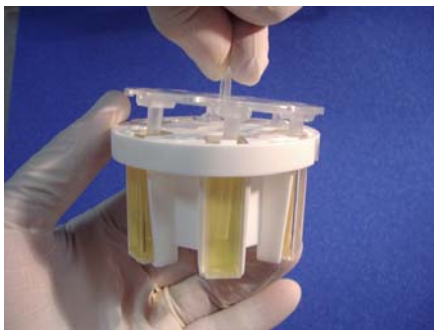


QwikLite 200

BIOSENSOR SYSTEM

Cartridge Prep Manual



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Planning

Planning – Before you begin any preparation, dosing, or testing procedures, make sure you have read all relevant instructions from Assure Controls, and that you have all materials necessary for testing. It is helpful to review all steps prior to testing to insure that you have all materials and processes in place for successful testing.

Materials

Collect all necessary items for testing. This includes:

Items Provided by Assure Controls with Purchase of Test Cartridge (QTC-100)

Purchase of each single Test Cartridge includes the following items. All items are necessary to complete a single test.

- Carousel (1)
- Snap top cover (1)
- Optical grade, methyl acrylic cuvettes (6)
- 50 mL conical mixing tube (1)
- 5 mL container of live cultures (*P. lunula* dinoflagellates)
- Commercial grade aquarium sea salt (for use with samples under 30 ppt)

When you purchase Test Cartridges, all CONTROL Test Cartridges are provided free of charge, at a ratio of (1) CONTROL per (5) samples to be tested.

Items Provided by Assure Controls with Purchase of QwikLite Accessories Kit (QAK-100)

Items in the QwikLite Accessory Kit are necessary for conducting tests with the QwikLite Biosensor Systems. The accessory kit serves as a useful accompaniment to the test and allows for fast and reliable testing.

- Light cycle box and timer (1)
- Refractometer (1) for salinity adjustment
- Precision Pipette (1)
- Precision Pipette tips (1 per test, 50 are included in Accessories Kit) - Additional sold separately

Items to be purchased separately by testing organization:

- Precision Pipette tip (1)

Many testing organizations will have their own pipettes and pipette tips which they will use for testing. For those organizations who purchase the QwikLite Accessories Kit, this kit includes a precision pipette, United Scientific Part # PVV-5000, and 50 pipette tips.

After use of the first 50 tips, it is recommended for the testing organization to purchase pipette tips separately. The correct pipette tip for United Scientific Pipette is United Scientific Part # T 5ML-N (<http://www.unitedsci.com/detail.php?id=1030>)

Light exposure during Dosing

Dosing is often conducted during a “Dark” phase of the test culture Light/Dark Cycle. In this circumstance, ambient light exposure should be limited as much as possible, but some limited exposure to light during Dosing is acceptable and will not interfere with testing.

Preparing CONTROL Test Cartridge

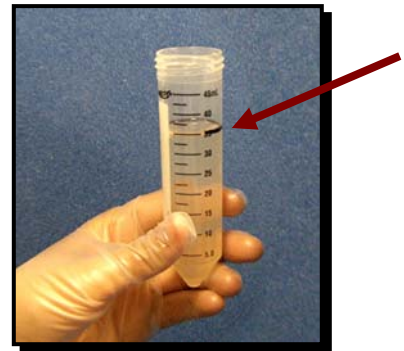
IMPORTANT SAFETY NOTE: Always wear gloves during all phases of cartridge preparation. Always wear eye protection and provide adequate ventilation when handling potentially harmful chemicals.

Tip: It is recommended to label the snap top covers of all Test Cartridges (CONTROLS and Samples) prior to Dosing using small circular labels (such as Avery 5472). Do not cover any agitation holes in the snap top cover when labeling.

NOTE: The following procedure is for making a single CONTROL. If your Testing Event will require multiple CONTROLS, we recommend that you prepare all Artificial Seawater in advance. Multiply # of CONTROLS by 35 mL, plus 10%, for the volume of bottled water to be salinity adjusted. Then follow the instructions below, multiplying salt as necessary for the number of CONTROLS.

Salinity Adjustment

1. Fill the conical tube with 35 mL of Crystal Geyser bottled water. Add 1.4 grams of salt (2 vials of .7 g sea salt from Assure Controls). Pipette 1-3 drops of Sample water on to the refractometer and measure salinity. Record salinity. Proceed to either “Fine Adjustment to Increase Salinity” or “Fine Adjustment to Decrease Salinity.”



Fine Adjustment to Increase Salinity

1. Enter current volume, current salinity, and desired salinity in to the “Not Salty Enough?” table in the Assure Controls salinity calculator. The Salinity Calculator will provide the weight of salt to be added in grams.

2. Add salt. Salt has been provided in quantities of approximately .7 grams, which is half the amount required to increase a common freshwater sample (0 ppt salinity) to a salinity of 33 ppt. Based on this weight, add the approximate weight of salt necessary to reach the desired salinity. You may need only a small bit for fine adjustment.



3. Pipette 1-3 drops of Sample water on to the refractometer and measure salinity. Record salinity. If the salinity is still too low, repeat Steps 2 and 3. If the salinity is now too high, proceed to Step 1 of “Decreasing Salinity.”

Fine Adjustment to Decrease Salinity

1. Enter current volume, current salinity, and desired salinity in to the “Too salty?” table in the Assure Controls salinity calculator. The Salinity Calculator will provide the volume of sample to be added in grams.

2. Add the volume of Sample indicated in Step 1. Pipettor may be helpful at this stage.

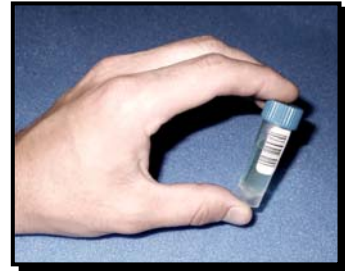
3. Pipette 1-3 drops of Sample water on to the refractometer and measure salinity. Repeat steps as necessary to achieve desired salinity.

After salinity adjustment, the conical tube may have a volume of than 35 mL. If so, use pipettor to remove the amount needed to obtain 35 mL.

NOTE: After salinity adjustment, you may have slightly more or less water than 35 mL. Use your pipettor to add or remove water as necessary, and re-check salinity if necessary. This must be done prior to adding culture.

Adding Culture to Artificial Seawater and Pipetting to Cartridge

1. Select a shipping vial of test culture. All shipping vials for each shipment include test culture in precisely measured 5 mL volumes of equal cell density; therefore any vial within the same shipment can be used for any CONTROL or sample.



2. Gently invert the shipping vial filled with test culture several times so that all floating material becomes suspended in an even, homogenous mixture. Create movement in the culture but do not shake vigorously.

4. Remove the lid on the vial of test culture and empty in to the conical tube which contains 35 mL Artificial Seawater.



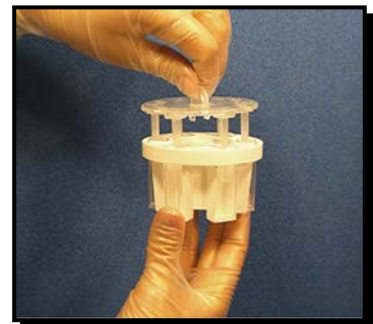
5. Prepare your pipettor so that it is set at 2 mL and has a fresh unused pipette tip.

6. For approximately 15 seconds, gently invert the conical tube so that all cells will be homogeneously (evenly) distributed. Hold one hand under the conical tube to prevent dropping while swirling. Create movement in the culture without vigorous shaking.

7. While the homogenous mixture remains in suspension, pipette 2 mL of solution from the conical mixing tube in to 3 cuvettes of the CONTROL cartridge. Cap the conical tube and repeat the swirl procedure from Step 6 before pipetting the final 3 cuvettes. This ensures that culture will not settle to the bottom of the conical tube during Dosing. Pipette from the same point in the conical tube for every cuvette.



8. Place the snap top cover on to the cartridge, and place in appropriate Light Dark Cycle Chamber.



Preparing Sample Test Cartridge – Salinity Adjustment

Salinity adjustment is required for all water samples below 31 ppt (freshwater and brackish water). If you are testing brackish water, or water of unknown salinity, check salinity prior to preparation and adjust salinity if necessary.

The acceptable overall salinity range for culture health and maintenance is 31-35 ppt. However, within a given Testing Event, the range of variation between CONTROLS and samples should be adjusted to within a range of 3 ppt. This will increase the consistency of experience between the cultures and help avoid variance in results. The following table provides examples of different salinity ranges and their acceptability for different functions.

Salinity Range	Acceptable for:
31-35 ppt	Acceptable for general cell maintenance
31-33 ppt	Acceptable range of variation within a given test
32-34 ppt	Acceptable range of variation within a given test
33-35 ppt	Acceptable range of variation within a given test
31-35 ppt	Not an acceptable range of variation within a given test – too much variation
32-35 ppt	Not an acceptable range of variation within a given test – too much variation

Salinity for all samples should be matched within a range of 3 ppt to the salinity of your CONTROL environment.

Example: CONTROL salinity is measured at 33 ppt. Salinity for All Samples tested in that CONTROL group should be adjusted to 32-34 ppt.

1. Fill the conical tube with 35 mL of Sample water. Save your Sample water as more volume of Sample may be needed. Pipette 1-3 drops of Sample water on to the refractometer and measure salinity. Record salinity. For most freshwater samples, salinity will be 0-1 ppt, and you can add 1.4 g of salt at this time (2 vials of .7 g sea salt from Assure Controls).



2. Proceed to either “Fine Adjustment to Increase Salinity” or “Fine Adjustment to Decrease Salinity” (Pages 2-3).

NOTE: After salinity adjustment, you may have slightly more or less water than 35 mL. Use your pipettor to add or remove water as necessary, and re-check salinity if necessary. This must be done prior to adding culture.

Preparing Sample Test Cartridge - Dosing

1. If you salinity adjusted, the conical tube may have slightly more or less water than 35 mL. If so, use pipettor to add or remove the amount needed to obtain 35 mL. Conical tubes from Assure Controls will have a black mark to annotate the 35 mL mark.

2. Select a shipping vial of test culture. All shipping vials for each shipment include test culture in precisely measured 5 mL volumes of equal cell density; therefore any vial within the same shipment can be used for any CONTROL or Sample.



3. Gently invert the shipping vial filled with test culture several times so that all floating material becomes suspended in an even, homogenous mixture. Create movement in the culture but do not shake vigorously.

4. Remove the lid on the vial of test culture and empty in to the conical tube.

5. Prepare your pipettor so that it is set at 2 mL and has a fresh unused pipette tip.

6. Gently invert the conical tube so that all cells will be homogeneously (evenly) distributed. Hold one hand under the conical tube to prevent dropping while swirling. Create movement in the culture without vigorous shaking.



7. While the homogenous mixture remains in suspension, pipette 2 mL of solution from the conical mixing tube in to 3 cuvettes of the first CONTROL cartridge. Cap the conical tube and repeat the swirl procedure from Step 6 before pipetting the final 3 cuvettes. This ensures that culture will not settle to the bottom of the conical tube during Dosing. Pipette from the same point in the conical tube for every cuvette.



8. Place the snap top cover on to the cartridge, and place in appropriate Light Dark Cycle Chamber.