Sessile Test Kit Instructions

- Product Description:

PBS

1. N.A.C.E. standard (TM0194-2014) Phosphate Buffer Solution (PBS)
2. **Physical Characteristics**: Clear
3. **Detection**: Used for converting sessile bacterial samples into planktonic samples. The Planktonic samples are then used to perform a serial dilution.

MPB

1. N.A.C.E. standard (TM0194-2004) Anaerobic Modified Postgate's B (MPB)
2. Anaerobic bacterial growth media, such as MPB, is extremely sensitive to oxygen exposure. Biotechnology Solutions' anaerobic bacterial growth media is manufactured inside custom built anaerobic chambers to ensure the highest quality bacterial growth media products.
3. **Physical Characteristics**: This media is white and opaque in color and contains no iron nail.
4. **Chemistry**: This media contains an oxygen scavenger that helps it maintain the anaerobic environment even upon introduction of an oxygenated sample. This media will turn pink for a few seconds when oxygen is introduced to the vial. Unlike API-RP38 this media does not contain an iron nail; it instead utilizes dissolved iron. MPB also contains extra carbon sources that facilitates the growth of certain strains of SRB thus making it more sensitive than API-RP38. A sulfate source is added to help mimic an environment conducive to SRB growth
5. **Detection**: Used for the enumeration of Sulfate Reducing Bacteria(SRB) in oil and gas systems, seawater, sediment, or water rich in decaying organic material.

PRD

1. N.A.C.E. standard (TM0194-2004) Phenol Red Dextrose Media
2. **Physical Characteristics**: Bright to Dark Red in Color
3. **Chemistry**: Contains proteins, sugars, and a pH indicator.
4. **Detection**: Used for the enumeration of total Acid Producing Bacteria(APB) as well as total General Heterotrophic Bacteria(GHB)

- Collecting a Sample:

1. Collect the sample in such a manner as to preclude contamination from external sources.
2. Time, date, temperature, and appearance of the sample should be recorded.
3. Using a sterile cotton swab, gently scrape the sessile sample area and place the solids into the sterile Phosphate Buffer Solution (PBS). The area being swabbed should be recorded (typically, a 1 cm^2 area is sampled). Break the used end of the wooden cotton swab off into the PBS solution and close the cap.
4. Once the sessile sample is in the PBS bottle, shake the PBS bottle thoroughly to evenly disperse the solids. Sonication can also be used to evenly disperse the solids.

- Sterilization: Wipe the rubber caps of the media vials with sterile alcohol pads.

- Inoculation:

MPB

1. Using a sterile disposable syringe, withdraw 1 mL of the sample from the PBS solution and inject it into the Modified Postgate's B (MPB) bottle #1 and discard syringe. Mix contents thoroughly by vigorously agitating the vial. Some bubbles may appear; this is normal.
2. Make sure to note changes in the vial upon inoculation that may cause a **False Positive**.

For additional information, please contact us at:
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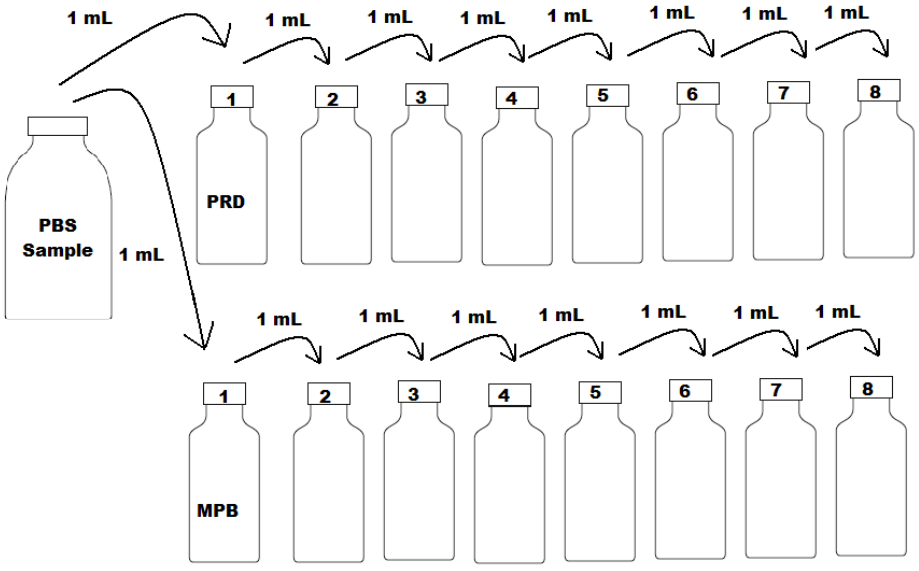
- **False Positive**: If the sample fluid injected into the vial contains a significant concentration of dissolved hydrogen sulfide, the first one to two vials in the dilution series may turn black. Vials should be recorded as false positives if these vials immediately develop a black precipitate upon inoculation. If you are not sure if the vial in question is positive for growth or simply a false positive, you can subculture the vial by performing a serial dilution using 1 mL from that vial.
- We recommend having a water analysis done so that you know what constituents and at what concentration the sample is made of.

3. With a new sterile syringe, withdraw 1 mL of solution from bottle #1 and inject it into bottle #2 and discard syringe. Mix contents thoroughly by vigorously agitating the vial.
4. Repeat this process for all the remaining dilution vials (#3 - #6).
5. Incubate the vials at the temperature at which the original sample was collected (± 2°C). N.A.C.E. standards call for 28-day incubation for SRB media.

PRD

6. Using a sterile disposable syringe, withdraw 1 mL of the sample from the PBS solution and inject it into the Phenol Red Dextrose (PRD) bottle #1 and discard syringe. Mix contents thoroughly by vigorously agitating the vial. Some bubbles may appear; this is normal.
7. Make sure to note changes in the vial upon inoculation that may cause a **False Positive**.
    - If the vials turn yellow immediately upon injection, the color change is due to the low pH of the sample fluids, not biogenic activity. If the questionable positive media is perfectly clear even though the yellow color change has taken place, this is likely due to contamination and not bacterial activity. If acid producing bacteria are present in the vial, turbidity (cloudiness) of the media will be apparent visually. If you are not sure if the first vial turned immediately upon inoculation or what the pH of the sample is, you can subculture the vial in question by performing a serial dilution using 1 mL from that vial. We recommend having a water analysis done so that you know what constituents and at what concentration make up the sample.
8. With a new sterile syringe, withdraw 1 mL of solution from bottle #1 and inject it into bottle #2 and discard syringe. Mix contents thoroughly by vigorously agitating the vial.
9. Repeat this process for all the remaining dilution vials (#3 - #6).
10. Incubate the vials at the temperature at which the original sample was collected (± 2°C). N.A.C.E. standards call for 14-day incubation for APB media.



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- Reading:

MPB

1. **Indicator for (SRB)**: As Sulfate Reducing Bacteria metabolize they reduce sulfate to sulfide. This sulfide then binds with the dissolved iron in the SRB media creating a black precipitate (Iron Sulfide). This black precipitate indicates that the vial is positive for SRB.
2. Record the number of positive vials.

PRD

1. **Indicator for (APB)**: Acid Producing Bacteria will drop the pH of the media causing a color change from red to yellow. The color change must also be accompanied by biomass or turbidity
2. **Indicator for (GHB)**: General Heterotrophic Bacteria can grow in this media as well but will not cause a color change from red to yellow. GHB can be identified in the vials by visually identifying turbidity or biomass accompanied by NO change in the red color of the media.
3. Record the number of positive vials.

- Disposal:

How should we dispose of Biotechnology Solutions' microbiological media waste?

All of BTS bacterial growth media products are considered to be non-hazardous materials. Media vials may be discarded according to your local, state and federal regulations. To find out more about these regulations please refer to the environmental, health and safety staff at your facility.

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