



## Serial Dilution Instructions Modified Postgate's B (MPB) FOR DETECTION OF SULFATE REDUCING BACTERIA

- Product Description:

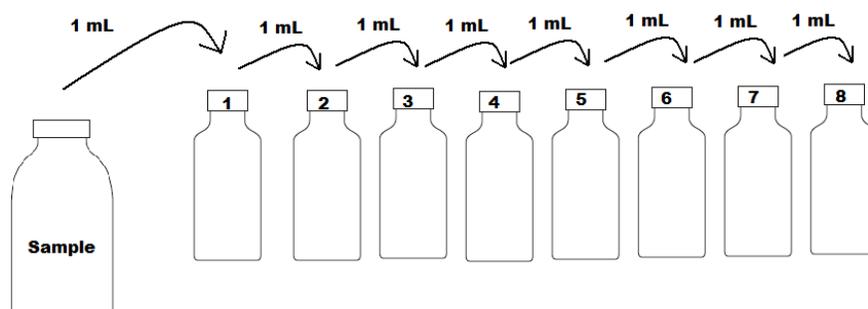
1. N.A.C.E. standard (TM0194-2004) Anaerobic Modified Postgate's B (MPB)
2. Anaerobic bacterial growth media, such as MPB, is extremely sensitive to oxygen exposure. Biotechnology Solutions' anaerobic bacterial growth media is manufactured inside custom built anaerobic chambers to ensure the highest 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.

- 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.

- Preparation:

1. Arrange selected media vials into "Dilution Series".
2. The selected media should approximate the conditions (Temp., TDS, etc.) of the sample water being tested to avoid the "shock" effect on the microbes.



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- Sterilization:

1. Wipe the rubber caps of the media vials with sterile alcohol pads.

- Inoculation:

1. Using a sterile disposable syringe, withdraw 1 mL of the sample and inject it into 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**.
  - **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 ( $\pm 2^{\circ}\text{C}$ ). N.A.C.E. standards call for 28-day incubation for SRB media and 14 days for APB media.

- Reading:

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.

- 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 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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