



## Serial Dilution Instructions for Iron Reducing Bacterial Broth (IRB) FOR DETECTION OF IRON REDUCING BACTERIA

- Product Description:

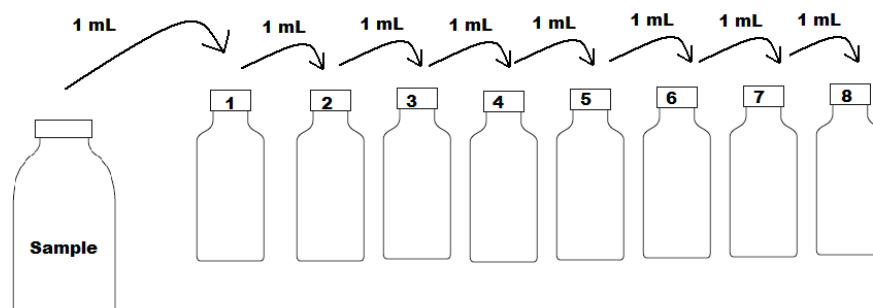
1. N.A.C.E. standard (TM0194-2014) Iron Reducing Bacterial Broth
2. **Physical Characteristics:** Slight brownish yellow color (Like dehydrated urine)
  - **Chemistry:** Both Magnesium sulfate and Ferric Citrate are present in the recipe which allow for a color change when Iron has been reduced.
3. **Detection:** Used for the enumeration of total Iron Reducing Bacteria(IRB).

- 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.



For additional information, please contact us at:  
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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. 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.
3. Repeat this process for all the remaining dilution vials (#3 - #6).
4. Incubate the vials at the temperature at which the original sample was collected ( $\pm 2^{\circ}\text{C}$ ). IRB media requires a 14-day incubation period.

- Reading:

1. **Indicator for (IRB):** As Iron Reducing Bacteria grow their metabolic process reduces Ferric to Ferrous. If the vial is positive for IRB you will notice turbidity and a color change to green(ish) Note: You may get a wide range of green colors.
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 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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