

Use of Methylene Blue with Oxyrase® BHI Broth

Purpose:

indicator. This procedure allows the user to visually confirm anaerobiosis in Oxyrase® BHI Broth with the use of Methylene Blue as an

Procedure:

- 1. Prepare a 0.05% (w/v) Methylene Blue Stock solution (0.5g/L).
- For every 5mL of broth add 0.01mL Methylene Blue Stock solution and then add 5-drops (0.4mL) Oxyrase® for Broth.
- 3. Incubate static for 15-20 minutes at 37°C.
- Broth will initially appear blue to green in color, and then revert to its natural color once dissolved oxygen is reduced to 0.1% or less
- S Oxygen will continue to dissolve at the liquid surface and produce a blue to green meniscus. Oxyrase® for Broth will actively remove this dissolved O2 but to prevent this, eliminate any head-space or replace atmosphere with anoxic gas

Expected results:

The pictures below show the expected results both before and after anaerobiosis is achieved.



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| Tube # | Tube # Description | Expected Results: Pre- | Expected Results: Post- |
|--------|---|------------------------|---------------------------------|
| | | Anaerobiosis | Anaerobiosis |
| 1 | BHI Broth Only (Control) | Brownish - Red | Brownish - Red (unchanged) |
| 2 | BHI Broth + Methylene Blue (Control) | Blue/Green to Brown | Blue/Green to Brown (unchanged) |
| ω | BHI Broth + Methylene Blue + Oxyrase® for Broth Blue/Green to Brown | Blue/Green to Brown | Brownish - Red |
| | | | |

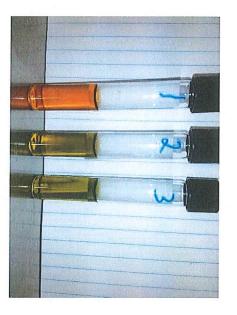


Figure 1: Pre-Anaerobiosis.

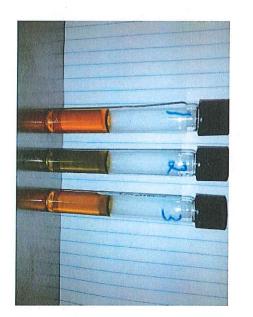


Figure 2: After 37C Incubation for 20 mins. Anaerobiosis achieved