



Use of Methylene Blue with Oxyrase® BHI Broth

Purpose:

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

Procedure:

1. Prepare a 0.05% (w/v) Methylene Blue Stock solution (0.5g/L).
2. 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.
4. 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.
5. 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 #	Description	Expected Results: Pre- Anaerobiosis	Expected Results: Post- 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)
3	BHI Broth + Methylene Blue + Oxryrase® for Broth	Blue/Green to Brown	Brownish - Red



Figure 1: Pre- Anaerobiosis.



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