METHYL RED REAGENT

- For in vitro use only - Catalogue No. RM65

Our Methyl Red (MR) Reagent is an indicator solution used to indicate the pH of the broth culture in the methyl red test.

The methyl red test is used to detect the ability of an organism to produce and maintain acid end products from glucose fermentation. The test is useful for differentiating among members of the *Enterobacteriaceae*, and is usually performed alongside the Voges-Proskauer (VP) test since both test are performed on cultures grown in MR-VP Broth.

The methyl red test is a quantitative test that measures the amount of acid produced by different bacterial species. All members of the *Enterobacteriaceae* can convert glucose to pyruvic acid by the Embden-Meyerhof pathway, but bacteria can further metabolize pyruvic acid by two different pathways.

Organisms metabolizing pyruvic acid by the mixed acid pathway will produce more acid end products, such as lactic acid and acetic acid, and maintain an acidic environment. The acidic pH produces a positive methyl red reaction and the color of the broth appears red or pink after the addition of Methyl Red Reagent.

Organisms metabolizing pyruvic acid by the butylene glycol pathway will produce neutral end products, such as acetoin and butanediol, which will raise the culture back to a neutral pH. The neutral pH results in a negative methyl red reaction, and the broth appears yellowish-orange after the addition of Methyl Red Reagent.

When using MR-VP Broth, the Voges-Proskauer test can be performed after 24 hours of incubation, but the methyl red test requires the broth be incubated a minimum of 48 hours prior to testing. Some researchers recommend a 3 to 5 day incubation period.

Formulation per 100 mL

Methyl Red	0.02 g
Ethyl Alcohol	60.0 mL
Sterile Deionized Water	40.0 mL

Recommended Procedure

Classical Procedure:

1. Inoculate a tube containing 5-mL of MR-VP broth (Dalynn TM85) with the organism of interest from a overnight culture grown on KIA, TSI Agar, MacConkey Agar, or Blood Agar.
2. Incubate for 24 hours at 35°C.
3. Remove 2.5-mL of the incubated broth to a separate tube for VP testing. (Refer to appropriate technical source for VP testing protocol)
4. Re-incubate the remaining 2.5-mL for an additional 1-4 days.
5. On the day of testing, allow sufficient time for Methyl Red Reagent to warm to room temperature prior to use.
6. Add 5 drops of Methyl Red Reagent to the tube. Shake the tube slightly and interpret the color result immediately.

Rapid Microtechnique Procedure:

1. From a stock solution of MR-VP Broth, aseptically pipette 0.5-mL aliquots into sterile test tubes (13x100-mm) just prior to use.
2. Pick the center of a single, well-isolated colony from an overnight culture grown on EMB Agar, MacConkey Agar, or Sheep Blood Agar and inoculate the broth.
3. Incubate tube at 35°C for 18 to 24 hours.
4. Allow sufficient time for Methyl Red Reagent to warm to room temperature prior to use.
5. Add one drop of Methyl Red Reagent. Shake the tube slightly and interpret the color result immediately.

Interpretation of Results

Positive: Pink or red color

Negative: Yellowish-orange color

- *The minimum incubation time for the MRVP Broth is 48 hours since the methyl red test is based on the complete metabolism of glucose and its major end product, pyruvic acid. Methyl red tests that are performed too early will result in false-positive results (classical)*

- *If the methyl red test results are inconclusive (orange) after 48 hours, continue incubation of the broth for an additional three days and retest the broth culture (classical)*

- *Methyl red is a pH indicator that has a range between 6.0 (yellow) and 4.4 (red)*

- *Avoid over inoculation of the MR-VP Broth. For optimal and reproducible results, standardize the inoculum used*

Quality Control

Organism	**Expected Results**	
Escherichia coli ATCC 25922	+ve	Red color
Klebsiella pneumoniae ATCC 13883	-ve	Yellow color

Storage and Shelf Life

Our Methyl Red Reagent should be stored at 4°C to 8°C. At this temperature it has a shelf life of 26 weeks from the date of manufacture.

References

1. Clark WM, Lubs HA. The differentiation of bacteria of the Colon-Aerogenes family by the use of indicators. J Infect Dis 1915; 17:160-73.
2. Barry AL, Bernsohn KL, Adams AB, Thrupp LD. Improved 18-hopur methyl red test. Appl Micro 1970; 20:866-70.
3. Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, Eds. Manual of clinical microbiology. 5th ed. Washington, DC: ASM, 1991.
4. Isenberg HD, Ed. Clinical microbiology procedures handbook. Washington, DC: ASM, 1992.
5. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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