

MIO Medium • Motility Indole Ornithine Medium

Intended Use

Motility Indole Ornithine (MIO) Medium is used to demonstrate motility, indole production and ornithine decarboxylase activity for the differentiation of *Enterobacteriaceae*.

Summary and Explanation

MIO Medium was formulated by Ederer and Clark¹ and Oberhofer and Hajkowski² for detection of motility, indole and ornithine decarboxylase production in one tube as an aid in the identification of members of the *Enterobacteriaceae* family.

Principles of the Procedure

Peptones, yeast extract and dextrose provide amino acids and other nitrogenous and carbonaceous substances, vitamins and minerals essential for bacterial metabolism. Motility can be read because of the semi-solid consistency of the medium. Organisms that possess the enzyme “tryptophanase” degrade the amino acid tryptophan to indolepyruvic acid, from which indole can be formed through deamination.³ When ornithine decarboxylase is present, the ornithine is decarboxylated to putrescine which causes a rise in the pH and corresponding color change of the bromcresol purple from yellow to purple.

Formula

Difco™ MIO Medium

Approximate Formula* Per Liter

Yeast Extract	3.0	g
Peptone	10.0	g
Tryptone	10.0	g
L-Ornithine HCl	5.0	g
Dextrose	1.0	g
Agar	2.0	g
Bromcresol Purple	0.02	g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To prepare the stored medium for use in motility studies, loosen caps, heat the medium to boiling and cool to room temperature prior to inoculation. Inoculate tubes of medium by a single stab to 1/4 inch from the bottom of the tube using growth from a

User Quality Control

Identity Specifications

Difco™ MIO Medium

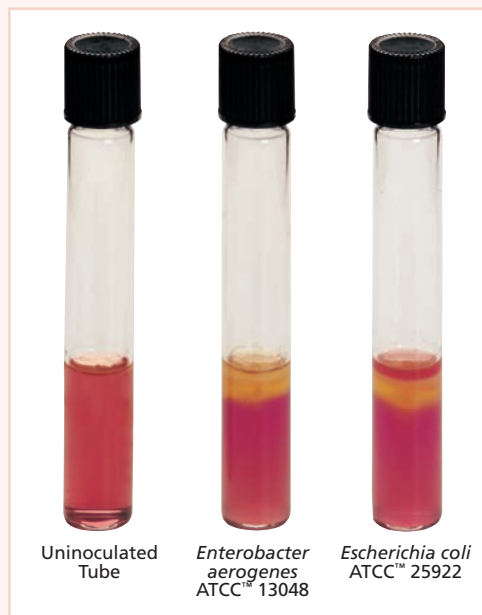
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in purified water upon boiling. Solution is purple, clear to slightly opalescent.
Prepared Appearance:	Purple, slightly opalescent, semi-solid.
Reaction of 3.1%	
Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

Difco™ MIO Medium

Prepare the medium per label directions. Inoculate with fresh cultures using an inoculating needle and incubate with caps loosened at 35 ± 2°C for 24-48 hours. Detect the presence of indole by the addition of 3-4 drops of Kovacs' Reagent.

ORGANISM	ATCC™	MOTILITY	INDOLE	ORNITHINE
<i>Enterobacter aerogenes</i>	13048	+	–	+
<i>Escherichia coli</i>	25922	+	+	+
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	13883	–	–	–
<i>Proteus mirabilis</i>	25933	+	–	+



primary isolation plate or other pure culture. Incubate all tubes for 18-24 hours at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere.

Expected Results

Read motility and decarboxylase activity prior to the addition of the reagent for the detection of indole production.

1. Motility is indicated by growth extending from the line of inoculation. Nonmotile organisms grow only along the line of inoculation.
2. Decarboxylation of ornithine is indicated by the development of a turbid purple to a faded yellow-purple color. A negative reaction is indicated by a yellow color.
3. Indole production is indicated by the formation of a pink to red color after the addition of three or four drops of Kovacs' reagent to the surface of the medium and gentle shaking. A negative reaction is indicated by the development of a yellow color.

Refer to appropriate texts for typical reactions produced by various members of the *Enterobacteriaceae*.⁴⁻⁶

References

1. Ederer and Clark. 1970. Appl. Microbiol. 2:849.
2. Oberhofer and Hajkowski. 1970. Am. J. Clin. Pathol. 54:720.
3. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ MIO Medium

BAM

Cat. No. 273520 Dehydrated – 500 g

BBL™ Motility Indole Ornithine Medium

BAM

Cat. No. 221517 Prepared Deeps (K Tubes), 5 mL – Pkg. of 10*
221518 Prepared Deeps (K Tubes), 5 mL – Ctn. of 100*

*Store at 2-8°C.