

MIO MEDIUM (7389)

Intended Use

MIO Medium is used for the differentiation of microorganisms on the basis of motility, ornithine decarboxylase activity, and indole production.

Product Summary and Explanation

Tests for indole production, motility, and ornithine decarboxylase activity play important roles in the identification of *Enterobacteriaceae*. Ederer and Clark¹ and Oberhofer and Hajkowski² developed MIO Medium, combining all three differentiating reactions in one medium. Ederer and Clark stressed the advantages of MIO Medium in their extensive study comparing cultural reactions of *Enterobacteriaceae* on MIO Medium with reactions on classic media.¹

Principles of the Procedure

The nitrogen, carbon, and amino acids sources are provided by Enzymatic Digest of Gelatin and Enzymatic Digest of Casein. Yeast Extract provides vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon. Dextrose is an energy source. The small concentration of agar is added to demonstrate motility. All *Enterobacteriaceae* ferment dextrose. Fermentation lowers the pH, causing MIO Medium to change from purple to yellow. If the organism possesses ornithine decarboxylase, L-Ornithine is decarboxylated to putrescine, causing the pH to increase and changing the color of the medium from yellow to purple. The pH indicator, Bromcresol Purple, facilitates detection of decarboxylase activity.

Formula / Liter

Enzymatic Digest of Gelatin	10 g
Enzymatic Digest of Casein	10 g
Yeast Extract.....	3 g
Dextrose.....	1 g
Bromcresol Purple	0.02 g
L-Ornithine	5 g
Agar	2 g

Final pH: 6.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 31g of the medium in one liter of purified water.
2. Heat with frequent agitation until dissolved completely.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and pale to light green-beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and purple.

Expected Cultural Response: Cultural response in MIO Medium were incubated aerobically at 35 ± 2°C and examined for growth after 18 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Reaction			
		Response	Motility	Indole	Ornithine
<i>Enterobacter aerogenes</i> ATCC® 13048	1 x 10 ⁸	Growth	Positive	Negative	Positive
<i>Escherichia coli</i> ATCC® 25922	1 x 10 ⁸	Growth	Positive	Positive	Positive
<i>Klebsiella pneumoniae</i> ATCC® 13883	1 x 10 ⁸	Growth	Negative	Negative	Negative
<i>Proteus mirabilis</i> ATCC® 12453	1 x 10 ⁸	Growth	Variable	Negative	Positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Using a wire, inoculate medium with stab motion to the bottom of the tube with isolated colonies.
2. Incubate with loose caps at $35 \pm 2^{\circ}\text{C}$ for 18 - 48 hours.
3. Examine tubes at 18 - 24 hours for growth, color change, and motility. Re-examine tubes at 40 - 48 hours.
4. Add 3 - 4 drops of Kovac's Reagent to each tube. Record as indole positive if a pink or red color appear, or as indole negative if there is no color change. Add Kovac's Reagent after determining motility and ornithine decarboxylase reactions.

Results

Motility is indicated by turbidity of the medium or growth extending from inoculating stab line. A purple color throughout the medium indicates a positive ornithine reaction. (The color may vary in intensity.) If the organism is ornithine negative, the medium is yellow. Indole is detected by adding Kovac's Reagent to the surface of the medium. A pink or red color indicates an indole-positive culture. Indole is produced from the tryptophane present in the medium.

Refer to appropriate references for complete identification of *Enterobacteriaceae*.³

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

MIO Medium	Code No.	7389A	500 g
		7389B	2 kg
		7389C	10 kg

References

1. **Ederer, G. M. and M. Clark.** 1970. Motility-Indole-Ornithine medium. *Appl Microbiol.* **2**:849.
2. **Oberhofer, T. R., and R. Hajkowski.** 1970. Evaluation of non-lactose-fermenting members of the *Klebsiella-Enterobacter-Serratia* Division. I. Biochemical characteristics. *Am. J. Clin. Pathol.* **54**:720.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover. (eds.)** 1995. *Manual of clinical microbiology*. 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.