PRODUCT SPECIFICATION SHEET

MIO Medium (Motility Indole Ornithine Medium) (DM618)

Intended Use

MIO Medium (Motility Indole Ornithine Medium) (DM618) is recomended for identification of *Enterobacteriaceae* on the basis of motility, indole production and ornithine decarboxylase activity.

Product Summary and Explanation

Motility, indole production and ornithine decarboxylation are routine biochemical tests employed during identification of *Enterobacteriaceae*. Motility can be verified microscopically (hanging drop) or macroscopically (tube method), where motility is observed as a diffused zone of growth extending out from the line of inoculation. Indole test is carried out to determine the ability of an organism to split indole from tryptophan present in casein enzymic hydrolysate, by the tryptophanase enzyme.[1,2] On reaction with Kovac's reagent, indole combines with the colour in the alcohol layer, which is visualized as a red ring (in the alcohol layer).[3] If the test organisms possess the specific decarboxylase enzyme, then ornithine is decarboxylated to putrescine, an amine, which results in a subsequent rise in the pH of the medium towards alkalinity. This causes the pH indicator bromocresol purple to change from purple to yellow colour. MIO (Motility Indole Ornithine Medium) was formulated by Ederer and Clark and evaluated by Oberhofer and Hajkowski for identification of Enterobacteriaceae, on the basis of motility, indole production and ornithine decarboxylation in a single tube.[4,5]

Principles of the Procedure

Casein enzymic hydrolysate and peptic digest of animal tissue provide amino acids, carbon and other nitrogenous substances required for growth of organisms. Yeast extract is the source of vitamin B complex. Dextrose is the fermentable carbohydrate. Organisms ferment dextrose to form acid, which causes the pH indicator bromocresol purple to change from purple to yellow. Organisms possessing ornithine decarboxylase enzyme, decarboxylate ornithine to putrescine which increases the pH making it alkaline, indicated by a colour change from yellow to purple throughout the medium. Decarboxylase negative reaction is indicated by yellow colour or yellow with a purple band near the top of the medium. Indole is produced from tryptophan present in casein enzymic hydrolysate. The indole produced combines with the aldehyde present in the Kovacs reagent to form a red complex.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Peptic digest of animal tissue	10.00
Yeast extract	3.00
L-Ornithine hydrochloride	5.00
Dextrose	1.00
Bromocresol purple	0.02
Agar	2.00
Final pH: 6.5 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 31.02 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense into tubes in 5 ml amounts.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool the tubes in an upright position.

PRODUCT SPECIFICATION SHEET

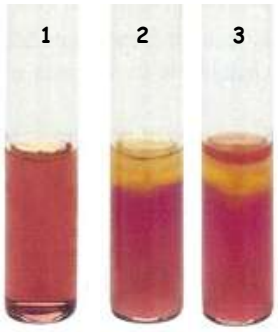
Quality Control Specifications

Dehydrated Appearance	Light yellow to pale green homogeneous free flowing powder
Prepared Medium	Purple coloured clear to slightly opalescent gel forms in tubes as butts
Reaction of 3.1% Solution	pH : 6.5 ± 0.2 at 25°C
Gel Strength	Semisolid, as comparable with 0.2% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation 35 - 37°C for 40-48 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Motility	Indole Production	Ornithine Decarboxylation
1.	*Escherichia coli ATCC 25922*	50 -100	good-luxuriant	positive, growth away from stabline causing turbidity	positive reaction, red ring at the interface of the medium	Positive reaction, purple colour
2.	*Enterobacter aerogenes ATCC 13048*	50 -100	good-luxuriant	positive, growth away from stabline causing turbidity	negative reaction	Positive reaction, purple colour
3.	*Klebsiella pneumoniae ATCC 13883*	50-100	good-luxuriant	negative, growth along the stabline, surrounding medium remains clear	negative reaction	Negative Reaction
4.	*Proteus mirabilis ATCC 25933*	50-100	good-luxuriant	motility is temperature dependent, it is more pronounced at 20°C and almost absent at 35°C	negative reaction	Positive reaction, purple colour

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



Motility Indole Ornithine Medium (DM618)

1. Control
2. *Enterobacter aerogenes ATCC 13048*
3. *Escherichia coli ATCC 25922*

Test Procedure

1. Using an inoculating needle, stab tubes (containing 5mL medium) with growth from an 18-24 hours pure culture.
2. Incubate the tubes at 35 - 37°C for 40-48 hours.
3. After incubation, examine tubes for evidence of lysine deaminase, motility, lysine decarboxylase reactions and after addition of Kovac's reagent, indole production.

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 stab line. Non motile organisms grow only along the stab line of inoculation

PRODUCT SPECIFICATION SHEET

2. Ornithine decarboxylase is indicated by a purple color throughout the medium. This color may vary in intensity and may be bleached out to a pale light color due to reduction of the indicator.
3. Ornithine-negative cultures produce a yellow color.
4. Add 3 or 4 drops of Kovac's Reagent (Cat. No. IR002) to the top of each tube. The appearance of a pink to red color in the reagent is interpreted as a positive indole test. A negative reaction is indicated by the development of a yellow color.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

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

Limitations of the Procedure

1. Kovacs reagent for indole should not be added until the final lysine deaminase, lysine decarboxylase and motility results have been interpreted.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : MIO Medium (Motility Indole Ornithine Medium).
Product Code : DM618
Available Pack sizes : 500gm

References

1. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
2. Ewing W. H., 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co.,Inc., New York.
3. MacFaddin J. F., 2000, Biochemical tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
4. Ederer G. M. and Clark M., 1970, Appl. Microbiol., 20:849.
5. Oberhofer J. R. and Hajkowski R., 1970, Am. J. Clin. Pathol., 54:726.

Further Information

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PRODUCT SPECIFICATION SHEET

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