



PRODUCT SPECIFICATION SHEET

Phenylalanine Malonate Broth (Shaw and Clarke Medium) (DM606)

Intended Use

Phenylalanine Malonate Broth (Shaw and Clarke Medium) (DM606) is recommended for differentiation of members of *Enterobacteriaceae* on the basis of their ability to utilize malonate and produce pyruvic acid from phenylalanine.

Product Summary and Explanation

The *Enterobacteriaceae* are a large family of Gram-negative bacteria that includes, along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella* and *Shigella*. Other disease-causing bacteria in this family include *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter*. Members of the *Enterobacteriaceae* can be trivially referred to as enterobacteria or "enteric bacteria", as several members occur in the intestines of animals, including humans, and some are pathogenic, causing disease in certain animal species. Members of *Enterobacteriaceae* are the most frequently encountered bacterial isolates recovered from clinical specimens. A battery of biochemical tests may be required for the definitive identification of the members of the *Enterobacteriaceae*.⁽¹⁾ Shaw and Clarke⁽²⁾ formulated this medium for differentiating gram-negative enteric bacteria on the basis of their ability to utilize malonate and produce pyruvic acid from phenylalanine.⁽³⁾

Principles of the Procedure

Phenylalanine Malonate Broth (Shaw and Clarke Medium) contains yeast extract in the medium provides nutrients and B complex vitamins to the organisms. Monopotassium and dipotassium phosphates help to buffer the medium. Bromothymol blue is the pH indicator. Sodium chloride provides the essential ions and hence maintains osmotic balance. Malonate is utilized by organisms like *Klebsiella* and *Salmonella arizonae*, which produces an alkaline reaction and thus change the colour of the medium from light green to dark blue due to the pH indicator bromothymol blue. If the organisms are malonate negative, the colour of the medium remains light green. Members of the group *Proteus* and *Providencia* are capable of deaminating phenylalanine to pyruvic acid and this reaction can be determined by the addition of few drops of 10% ferric chloride dissolved in acidified distilled water to a freshly grown culture. Due to production of pyruvic acid from phenylalanine a deep green colour is formed. The results for malonate utilization should be read before adding ferric chloride solution to the test tube, to detect phenylalanine deamination.⁽⁴⁾

Formula / Liter

Ingredients	Gms / Liter
Yeast extract	1.00
Sodium malonate	3.00
DL-Phenylalanine	2.00
Ammonium sulphate	2.00
Dipotassium phosphate	0.60
Monopotassium phosphate	0.40
Sodium chloride`	2.00
Bromo thymol blue	0.025
Final pH: 6.3 ± 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.





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Directions

1. Suspend 11.03 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense in tubes.
4. Autoclave at 115°C, for 10 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light green homogeneous free flowing powder
Prepared Medium	Yellowish green coloured clear solution without any precipitate
Reaction of 1.1% solution	pH 6.3 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Malonate	Phenylalanine
1.	<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	negative reaction	negative reaction
2.	<i>Klebsiella pneumonia</i> ATCC 13883	50-100	good-luxuriant	positive reaction, dark blue colour	negative reaction
3.	<i>Proteus mirabilis</i> ATCC 25933	50-100	good-luxuriant	negative reaction	positive reaction, green colouration after addition of 10% ferric chloride
4.	<i>Providencia alcalifaciens</i> ATCC 9886	50-100	good-luxuriant	negative reaction	positive reaction, green colouration after addition of 10% ferric chloride
5.	<i>Salmonella Arizonae</i> ATCC 13314	50-100	good-luxuriant	positive reaction, dark blue colour	negative reaction
6.	<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	negative reaction	negative reaction

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

Refer to appropriate references and standard test procedures for interpretation of results.

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.





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Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Phenylalanine Malonate Broth (Shaw and Clarke Medium)

Product Code : DM606

Available Pack sizes : 100gm

References

1. Koneman E. W., Allen S. D., Janda W.M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4 th Ed., J. B. Lippincott Company.
2. Shaw C. and Clarke, 1955, J. Gen. Microbiol., 13:155.
3. Collee J.G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

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Unit 38/39, Kalpataru Industrial Estate,
Near Runwal Estate, Behind 'R-Mall' ,Ghodbunder Raod,
Thane (W) - 400607. M.S. INDIA.

Ph: +91-22-25895505, 4760, Cell: 9320126789.

Email: micromaster@micromasterlab.com
sales@micromasterlab.com

Prepared By	Checked By	Approved By
 01.01.2018	 01.01.2018	 01.01.2018
Microbiologist	Head Quality Control	Head Quality Assurance

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