# Technical Information

## Phenylalanine Malonate Broth (Shaw and Clarke Medium)

## Product Code: DM 1781

**Application:** Phenylalanine Malonate Broth is used for the differentiation of members of *Enterobacteriaceae* on the basis of their ability to utilize malonate and produce pyruvic acid from phenylalanine.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Yeast extract | 1.000 |
| Sodium malonate | 3.000 |
| DL-Phenylalanine | 2.000 |
| Ammonium sulphate | 2.000 |
| Dipotassium phosphate | 0.600 |
| Monopotassium phosphate | 0.400 |
| Sodium chloride | 2.000 |
| Bromo thymol blue | 0.025 |
| Final pH ( at 25°C) | 6.3±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Principle & Interpretation

The term enteric bacteria is generally used in reference to organisms of the Family *Enterobacteriaceae,* many members of which occur in the enteric tract of humans and animals. Members of *Enterobacteriaceae* are the most commonly isolated bacterial recovered from clinical specimens. Definitive identification of the members of the *Enterobacteriaceae* may require a battery of biochemical tests [(1)]. This medium is prepared according to the formulation developed by Shaw and Clarke [(2)] for differentiating gram-negative enteric bacteria on the basis of their ability to utilize malonate and produce pyruvic acid from phenylalanine [(4)].

Yeast extract in the medium supplies nutrients to the organisms while phosphates buffer the medium. Bromothymol blue is the pH indicator. Sodium chloride maintains osmotic balance. Organisms like *Klebsiella* and *Salmonella arizonae,* which are capable of, utilizing malonate, produce an alkaline reaction and thus change the colour of the medium from light green to dark blue indicated by the pH indicator bromothymol blue. The colour of the medium remains light green if the organisms are malonate negative. Members of the group *Proteus* and *Providencia* are capable of deaminating phenylalanine to pyruvic acid. 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. Deep green colour is formed due to production of pyruvic acid from phenylalanine. Malonate utilization results should be read before adding ferric chloride solution to the test tube, to detect phenylalanine deamination [(3)].

## Methodology

Suspend 11.03 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 115°C for 10 minutes.

## Quality Control

**Physical Appearance**
Light yellow to light green homogeneous free flowing powder
**Colour and Clarity of prepared medium**
Yellowish green coloured clear solution without any preciipitate
**Reaction**
Reaction of 1.1% w/v aqueous solution at 25°C. pH : 6.3±0.2
**pH pH Range** 6.10-6.50
**Cultural Response/ characteristices**

DM 1781: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

| Organism | Inoculum (CFU) | Growth | Malonate | Phenylalanline |
|---|---|---|---|---|
| Escherichia coli ATCC 25922 | 50-100 | luxuriant | negative reaction | negative reaction |
| Klebsiella pneumoniae ATCC 13883 | 50-100 | luxuriant | positive reaction, dark blue colour | negative reaction |
| Proteus mirabilis ATCC 25933 | 50-100 | luxuriant | negative reaction | positive reaction, green colouration after addition of 10% ferric chloride |
| Providencia alcalifaciens ATCC 9886 | 50-100 | luxuriant | negative reaction | positive reaction, green colouration after addition of 10% ferric chloride |
| Salmonella Arizonae ATCC 13314 | 50-100 | luxuriant | positive reaction, dark blue colour | negative reaction |
| Salmonella Typhimurium ATCC 14028 | 50-100 | luxuriant | negative reaction | negative reaction |

## Storage and Shelf Life

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
**Prepared Media**: 2-8° in sealable plastic bags for 2-5 days.

## Further Reading

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. Lippinccott Company
2. Shaw C. and Clarke, 1955, J. Gen. Microbiol., 13:155.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
4. Collee J.G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone

## Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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