



Mineral Modified Glutamate Medium Base (Double Strength) (Twin Pack)

M643

Mineral Modified Glutamate Medium (Double Strength) is used for the enumeration of coliform bacteria in water and wastewater samples.

Composition**

| Ingredients | Gms / Litre |
|-------------------------|-------------|
| Part A | - |
| Lactose | 20.000 |
| Dipotassium phosphate | 1.800 |
| Sodium formate | 0.500 |
| L-Cystine | 0.040 |
| L-Aspartic acid | 0.048 |
| L-Arginine | 0.040 |
| Thiamine | 0.002 |
| Nicotinic acid | 0.002 |
| Pantothenic acid | 0.002 |
| Magnesium sulphate | 0.200 |
| Ferric ammonium citrate | 0.020 |
| Calcium chloride | 0.020 |
| Bromocresol purple | 0.020 |
| Part B | - |
| Sodium glutamate | 12.700 |
| Final pH (at 25°C) | 6.7±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22.69 grams of Part A and 12.7 grams of Part B in 1000ml distilled water containing 5 grams of ammonium chloride. Heat if necessary to dissolve the medium completely. Dispense in tubes containing inverted Durhams tubes. Sterilize by autoclaving at 115°C for 10 minutes. For single strength medium use 11.35 grams of Part A and 6.35 grams of Part B in 1000 ml distilled water containing 2.5 grams ammonium chloride.

Principle And Interpretation

MacConkey Broth was originally used for enumeration of coliform bacteria in water. However Folpmers (1) described a glutamic acid based chemically defined medium for the same. This glutamate-containing medium was later modified by Gray (2), by the addition of lactose, which gave less false positive results when compared to MacConkey Broth. To improve gas production in the medium, it was supplemented with ammonium chloride (3). Mineral Modified Glutamate Medium is used for the enumeration of coliform bacteria in water. This medium gave better results than MacConkey Broth (with less false positives) while testing water samples, both chlorinated and unchlorinated (4). Mineral Modified Glutamate Medium is superior to other media, in testing for coliforms and *Escherichia coli* because it initiates faster growth. This medium is recommended by APHA (7) and is also used to enrich coliform organisms present in cheese and meat (5).

This medium contains a variety of nutrients including salts, amino acids and vitamins. Lactose is the fermentable carbohydrate and bromo cresol purple is the pH indicator. Because of the nutrients, this medium is superior for enumerating coliforms in water and wastewater as it satisfies most of the nutritional requirements of coliforms.

If the test water sample is supposedly of good quality, inoculate 50 ml water sample in 50 ml of medium. Also inoculate 5 tubes of 10 ml Mineral Modified Glutamate Medium Base (M643), each with 10 ml water sample. If water sample is more polluted, inoculate 5 tubes of 5 ml of single strength medium each with 1 ml of a 1: 10 dilution of the sample. Incubate the tubes at

35-37°C for 18-24 hours. Tubes showing yellow colouration, due to acid production and gas formation, (bubbles trapped in Durhams tubes) are presumptively positive. These should be further confirmed by inoculating into Brilliant Green Bile Broth 2% (M121) and by performing biochemical tests.

MPN per 100 ml of water sample can be calculated by referring to MPN Chart (6).

Quality Control

Appearance

Part A : Light yellow to green homogeneous free flowing powder Part B : White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured clear solution without any precipitate

Reaction

Reaction of the medium (mixture of 2.27% w/v Part A + 1.27% w/v Part B + 0.5% w/v ammonium chloride) at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Cultural Response

| Organism | Inoculum (CFU) | Growth | Acid | Gas |
|--|-------------------|-----------|-------------------|-------------------|
| Cultural Response | | | | |
| <i>Enterobacter aerogenes</i> ATCC 13048 | 50-100 | luxuriant | positive reaction | negative reaction |
| <i>Enterococcus faecalis</i> ATCC 29212 | >=10 ³ | inhibited | | |
| <i>Escherichia coli</i> ATCC 25922 | 50-100 | luxuriant | positive reaction | positive reaction |
| <i>Salmonella Typhi</i> ATCC 6539 | 50-100 | luxuriant | negative reaction | negative reaction |
| <i>Shigella flexneri</i> ATCC 12022 | 50-100 | luxuriant | negative reaction | negative reaction |
| <i>Staphylococcus aureus</i> ATCC 25923 | >=10 ³ | inhibited | | |

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

- Folpmers T., 1948, J. Microbiol. Serol., 14:58-64
- Gray R. D., 1959, J. Hyg. Camb. 57:249.
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- P. H. L. S. Standing Committee on the Bacteriological Examination of Water Supplies, 1968, J. Hyg. Camb. 65:67.
- Abbyss J. S., Wilson J. M., Blood R. M. and Jarvis B., 1981, J. Appl. Bacteriol., 51:121.
- Department of Environment, Health and Social Security and P. H. L. S., 1982, The Bacteriological Examination of Water Supplies, Report on Public Health and Medical Subjects No. 71, H.M.S.O., London, England
- Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed. American Public Health Association, Washington, D.C.

Revision : 2 / 2015

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