

**MINERAL MODIFIED GLUTAMATE MEDIUM BASE (DOUBLE STRENGTH)
(DOUBLE PACK)**

TM 1245

For the enumeration of coliform bacteria in water and wastewater

Composition

Ingredients	Gms/Ltr.
Part I	
Lactose	20.00
Dipotassium phosphate	1.80
Sodium formate	0.50
L-cysteine	0.04
L-aspartic acid	0.048
L-arginine	0.040
Thiamine	0.002
Nicotinic acid	0.002
Pantothenic acid	0.002
Magnesium sulphate	0.20
Ferric ammonium citrate	0.020
Calcium chloride	0.020
Bromocresol purple	0.020
Part II	
Sodium glutamate	12.70

* Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight.

Instructions for use

Dissolve 22.70gms (Part I) and 12.70 (Part II) in 1000ml of distilled water, containing 5gm of ammonium chloride. Gently heat to boiling with gentle swirling and dissolve the medium completely. Dispense into tubes containing inverted Durham's tubes. Sterilize by autoclaving at 10psi (115°C) for 10 minutes. Cool to 45-50°C before use.

Appearance: Purple colour, clear solution

pH (at 25°C): 6.7 ± 0.2

Principle

MINERAL MODIFIED GLUTAMATE MEDIUM BASE is used for the enumeration of coliform bacteria in water and wastewater. This medium was modified by Gray, by the addition of lactose, which gave less false results on comparing with MacConkey Broth (TM 529). Sodium formate is the basis of the medium for the enumeration of coliform organisms in water. Lactose is the source of carbohydrates. The addition of vitamins, aminoacids and Magnesium sulphate to increase fermentation, whereas the addition of Ferric ammonium citrate permits increase of gas production. Dipotassium phosphate is a buffering agent. The

PRODUCT DATA SHEET

Bromocresol purple is a pH indicator. The addition of ammonium chloride improves the gas production in the medium. This media contains a variety of nutrients including amino acids, vitamins. Lactose is the fermentable carbohydrate source in the medium. For inoculation: Use the MPN technique. With 10ml and 50ml of sample add to equal volumes of double strength medium. With 1ml volumes of sample add to 5ml of single strength medium. Ensure the Durham tube is free of bubbles. These should be further confirmed by inoculating into Brilliant Green Bile Broth 2% (TM 365) and by performing biochemical tests. MPN per 100ml of water sample can be calculated by referring to MPN Chart. Incubation time period 37°C for 18 - 24 hours aerobically. Observation of Tubes showing the production of acid (medium turns yellow) and gas in the Durham's tube are considered presumptive positive. Each presumptive positive tube should be subcultured to Brilliant Green Bile Broth 2% (TM 365) with Durham tube and incubated at 44°C for 24 hours and examined for gas production. A tube of Tryptone Water (TM 392) should also be inoculated and incubated at 44°C for 24 hours for the production of indole. The production at 44°C of gas from lactose and the formation of indole are evidence of *E. coli*.

Interpretation

Cultural characteristics observed after inoculating (10^4 - 10^5 CFU/ml), on incubation at 35°C for 18 - 48 hours.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth	Acid/ Gas
<i>Escherichia coli</i>	25922	10^4 - 10^5	Good	+ / +
<i>Escherichia coli</i>	23724	10^4 - 10^5	Good	+ / +
<i>Enterobacter aerogenes</i>	13048	10^4 - 10^5	Good	+ / -
<i>Salmonella typhi</i>	6539	10^4 - 10^5	Good	- / -
<i>Staphylococcus aureus</i>	25923	10^4 - 10^5	Inhibited	-----

References

1. Abbiss J. S., Wilson J. M., Blood R. M. and Jarvis B., J. Appl. Bacteriol., 51:121. (1981).
2. Gray R. D., J. Hyg. Camb. 57:249. (1959).
3. Gray R. D., J. Hyg. Camb., 62:495. (1964,).
4. P. H. L. S. Standing Committee on the Bacteriological Examination of Water Supplies, J. Hyg. Camb. 65:67. (1968).