

Minerals Modified Glutamate Broth (MMBG) ISO

Cat. 1365

for the enumeration of β -glucuronidase-positive *Escherichia coli*.

Practical information

Applications	Categories
Selective enumeration	<i>Escherichia coli</i>
Selective enrichment	<i>Escherichia coli</i>

Industry: Water / Food

Regulations: ISO 11133 / ISO 16649

Principles and uses

Minerals Modified Glutamate Broth (MMBG) ISO is recommended by ISO 16649-3 for enumeration of *E.coli* using the most probable number Method (MPN). MMBG Broth is also an alternative broth used for the presumptive identification of coliforms in water.

In comparison to other media, this broth is better as it gives fewer false positive results. Sodium glutamate and sodium formate are 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 heptahydrate magnesium sulfate increase fermentation, whereas the addition of ferric ammonium citrate permits increase of gas production. The bromocresol purple is a pH indicator.

Formula in g/L

Bromocresol purple	0,01	Dipotassium phosphate	0,9
Ferric ammonium citrate	0,01	Lactose	10
L-Arginine	0,02	L-Cystine	0,02
Magnesium sulfate heptahydrated	0,1	Thiamine	0,001
Sodium glutamate	6,35	Sodium formate	0,25
L (-) aspartic acid	0,024	Calcium chloride dihydrate	0,01
Panthenic acid	0,001	Nicotinic acid	0,001

Preparation

Suspend 17,7 grams of the medium in one liter of distilled water. Add 2,5 grams of Ammonium Chloride. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 116 °C for 10 minutes.

Instructions for use

Enumeration of β -glucuronidase-positive *Escherichia coli* according to ISO 16649:

- Inoculate three tubes of a single strength and three tubes of a double strength medium.
- The larger volumes of sample (10 ml) are added to equal volumes of double strength medium, whereas the 1 ml volumes (or dilution of them) are added to 5 ml of single strength medium.
- Incubate the tubes at 37±1 °C and examine after 24±2 hours, for the acid production (lactose fermentation).
- Each tube of selective enrichment medium showing acid production (yellow color), is subcultured to TBX Chromogenic Agar (Cat. 1151).
- Incubate the TBX plates at 44±1 °C for 22±2 hours.
- The presence of characteristic blue colonies on TBX indicated the presence of *Escherichia coli* in the original MMBG tube.

* Those strains of *Escherichia coli* that do not grow at 44°C and, in particular, those that are β -glucuronide negative, such as *Escherichia coli* O0157: H7, will not be detected

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	White with black particles	Clear purple	6,7 ± 0,1

Microbiological test

According to ISO 11133:

Incubation conditions: Productivity, Selectivity (37±1 °C / 24±2 h).

Inoculation conditions: Productivity qualitative (<=100 CFU) / Selectivity (10⁴-10⁶ CFU).

Microorganisms	Specification	Characteristic reaction
Escherichia coli ATCC 25922	Acid production	Colour change to yellow
Enterococcus faecalis ATCC 29212	Total inhibition	
Escherichia coli ATCC 8739	Acid production	Colour change to yellow

Storage

Temp. Min.:2 °C

Temp. Max.:25 °C

Bibliography

ISO 16649-3 Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of β-glucuronidase-positive Escherichia coli- Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide.

Departments of the Environment, Health & Social Security, and P.H.L.S. 1982. The bacteriological examination of drinking water supplies. Report on public Health and Medical Subjects No. 71., H.M.S.O., London, England.