EO Labs
Microbiological Culture Media

Instructions for Use

REF - KM0213 **Minerals Modified Glutamate Medium**

Dehydrated Culture Media

1. Intended Use

KM0213 Minerals Modified Glutamate Medium is a selective enrichment medium for the isolation of coliforms from water, food, and environmental samples.

2. Composition*

Ingredient	g/L
Lactose	20.0
Dipotassium hydrogen phosphate	1.8
Sodium formate	0.5
L-cysteine	0.04
L-aspartic acid	0.048
L-arginine	0.04
Thiamine	0.002
Nicotinic acid	0.002
Pantothenic acid	0.02
Magnesium sulphate	0.02
Ferric ammonium citrate	0.02
Calcium chloride	0.02
Bromocresol purple	0.02

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Coliforms are facultatively anaerobic, Gram-negative bacteria that are part of the Enterobacteriaceae. They are oxidase-negative, can ferment and produce gas from lactose when grown at 37°C, and have β-galactosidase activity. In addition to positive results for these tests, *Escherichia coli* can be further distinguished by the capability to grow at 44°C and to produce indole from tryptophan.

Originally intended for enumeration of coliforms in water samples, a medium containing glutamic acid was devised by Folpmers [(1)] and further developed by Gray [(2)] as a potential replacement for MacConkey Broth. Later, Gray modified the medium to incorporate a variety of minerals promoting improved lactose fermentation by coliforms and *Escherichia coli* [(3)]. Minerals Modified Glutamate Medium is suitable for the enrichment of low levels of coliforms and recovery of chlorine-damaged bacteria [(4)].

Minerals Modified Glutamate Medium is recommended by the Standing Committee of Analysts [(5)] and International Organization for Standardization [(4)]. It is tested in accordance with ISO 11133:2014 [(6)].

4. Principle

Lactose is fermented to provide energy for bacterial growth. Acid produced during this process changes the colour of the medium from purple to yellow indicating the presence of lactose fermenting bacteria. Bromocresol purple is the pH indicator. Amino acids and vitamins are essential nutrients for the growth of coliforms and metal ions improve the fermentation of lactose. Dipotassium hydrogen phosphate is a buffer to stabilise pH but is present at a low level to allow fermentation derived colour change in the medium. A double strength preparation of the medium (see Section 5) may be used to allow the addition of an equal volume of sample without compromising medium utility.

5. Preparation Instructions

For the preparation of double strength* medium:

Suspend 22.69g of dehydrated culture medium (KM0213) in 1 litre deionised/purified water.
Add 5g of ammonium chloride and 12.7g of L-glutamic acid sodium salt.
Heat with frequent agitation and boil for one minute to completely dissolve the powder.
Dispense specified volume into appropriate containers; an inverted Durham tube may be added to visualise gas production.
Autoclave to sterilise at 115°C for 10 minutes before cooling in a water bath.

*For single strength medium, use half of the above stated amounts in 1 litre of deionised/purified water.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Purple fine powder	Purple liquid
pH	N/A	6.7 ± 0.2

7. Materials Provided

KM0213 is available in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0213-500G-500	1 x 500g Dehydrated Culture Medium Tub
KM0213-5KG-5000	1 x 5kg Dehydrated Culture Medium Tub
KM0213-10KG-10000	1 x 10kg Dehydrated Culture Medium Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

Additional ingredients for preparation of the medium (ammonium chloride and L-glutamic acid sodium salt) are not supplied by E&O Laboratories.

9. Specimens

KM0213 Minerals Modified Glutamate Medium is suitable for the testing of the following specimens:

- Water: drinking water, ground/river/sea water, sewage, and effluents.
- Food and Environmental: products for human consumption, animal feed and environmental samples.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Follow local, national, or international guidelines.

Allow medium to come to room temperature.

Water Specimens [5]

According to MoDW Part 4 [5], determine whether the specimen requires serial dilution prior to inoculation. Determine the volume of specimen to be added to the prepared medium.

- Process the water sample as required by relevant guidelines.
- Inoculate directly into the medium.
- Incubate the broths at 37 ± 1°C for 24-48 hours. Visual colour checks should be performed after 24-hour and 48-hour incubation.
- Presumptive coliforms should be subcultured into bottled media products such as E&O BM0860 Peptone Water with Lactose (Andrade's) and E&O BM0610 Tryptone Water for further confirmatory tests.

Food/Environment Specimens [4]

- Process the food/environmental samples as required by local guidelines.
- Inoculate directly into the medium.
- Incubate the broths at 37 ± 1°C for 24 ± 2 hours. Visual colour checks should be performed at 24 hours.
- Presumptive coliforms should be subcultured onto a poured plate medium containing 5-bromo-4-chloro-3-indolyl-β-D-glucuronide such as E&O PP1134 Tryptone Bile X-Glucuronide Agar.

After incubation, examine broths for colour change from purple to yellow indicating positive lactose fermentation. Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Results
E. coli (NCTC 12241)	37 ± 1°C for 24 ± 2 hours	Broth: Growth, yellow with acid and gas production.
E. coli (NCTC 12923)	37 ± 1°C for 24 ± 2 hours	Broth: Growth, yellow with acid and gas production.
K. aerogenes (NCTC 10006)	37 ± 1°C for 24 ± 2 hours	Broth: Growth, yellow with acid and gas production.
P. aeruginosa (NCTC 12903)	37 ± 1°C for 24 ± 2 hours Subcultured onto E&O PP0470 MacConkey without Salt Agar and incubated at 37 ± 1°C for 18-24 hours.	Broth: Growth, purple with no acid or gas production.
E. faecalis (NCTC 775)	37 ± 1°C for 24 ± 2 hours	Broth: Inhibited, purple with no acid or gas production.
E. faecalis (NCTC 12697)	37 ± 1°C for 24 ± 2 hours	Broth: Inhibited, purple with no acid or gas production.

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify the performance of KM0213 Minerals Modified Glutamate Medium, dehydrated culture medium samples were used to prepare Minerals Modified Glutamate Medium (Double Strength) and tested to assess turbidity, colour change, and recovery level. Prepared samples were inoculated and incubated at 37 ± 1°C for 24 ± 2 hours, then 1µl of the productivity broth was subcultured onto PP0470 MacConkey without Salt Agar and incubated at 37 ± 1°C for 18-24 hours. All samples of prepared medium produced expected colour change, recovery, and morphology of the required test organisms: *Escherichia coli* (NCTC 12241), *Escherichia coli* (NCTC 12923), *Klebsiella aerogenes* (NCTC 10006) and *Pseudomonas aeruginosa* (NCTC 12903), and no recovery of the non-target test organisms: *Enterococcus faecalis* (NCTC 775) and *Enterococcus faecalis* (NCTC 12697). Therefore, it can be concluded that KM0213 Minerals Modified Glutamate Medium, meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Additional biochemical, serological, or molecular tests should be performed on sub-culture isolates to confirm identity.
- Due to natural variation, some strains may grow poorly in this medium.
- Low levels of coliforms (lactose fermenters) may change the colour of the medium from purple to grey rather than yellow. Subculture should still be carried out.
- Some species of *Enterobacter* and *Klebsiella* may take longer to produce the yellow colour due to delayed lactose fermentation.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSSH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to KM0213 Material Safety Data Sheet.

15. Storage conditions and Shelf life

Store KM0213 dehydrated culture medium in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to date of expiry shown on the product label.

Dispose of in accordance with local and national authority requirements. Do not use if the dehydrated culture medium is not free-flowing, homogenous or the specified colour. Additionally, do not use the medium if it has been stored inappropriately or the packaging has been damaged.

Do not use prepared medium if it is contaminated, discoloured, or a precipitate is present. Additionally, do not use prepared medium if it has been stored inappropriately.

Dehydrated culture media does not need to be used all at once; it may be resealed and stored according to label instructions.

16. References

1. Folpmers, T. (1948) Is it justified to use Lactose Broth for the detection of *Bact. coli* in the presumptive test of routine water analysis? Leeuwenhoek ned. Tijdschr. 14: 58.
2. Gray, R.D. (1959) Formate lactose glutamate: a chemically defined medium as a possible substitute for MacConkey Broth in the presumptive coliform examination of water. J. Hyg. Camb., 57(3):249-265.
3. Gray, R.D. (1964) An improved formate lactose glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water. J Hyg (Lond). 62(4):495-508.
4. International Organization for Standardization (2015) 16649-3:2015 Microbiology of the food chain – Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* – Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. Geneva, ISO.
5. The Microbiology of Drinking Water (2020) Part 4 –Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including E. coli O157:H7). Standing Committee of Analysts, Environment Agency, Nottingham, UK.
6. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media. Geneva, ISO.

Version History*

001 21/06/23 - New Document Created

002 12/02/24 - Update sections 11/12 with *E. coli* NCTC 12923 and *E. faecalis* NCTC 775.

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com

EC REP Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00

TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code		Manufacturer	Use by
Temperature limitation	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place