Instructions for Use

REF - KM0125 **MacConkey Broth (USP)**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0125 MacConkey Broth (USP) is a selective medium for the enrichment of Enterobacteriaceae and the detection of *Escherichia coli in* water, milk, and other products.

2. Composition*

Ingredient	g/L
Pancreatic digest of gelatine	20.0
Lactose	10.0
Oxbile	5.0
Bromocresol purple	0.01

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

MacConkey Broth (USP) is a modification of the original bile salt broth recommended by MacConkey; since then, the neutral red has been replaced with the less inhibitory bromocresol purple, and bile salts replaced the original sodium taurocholate. MacConkey Broth (USP) can also be used for pre-enrichment of Gram-negative, lactose-fermenting bacilli in water and foods. This formulation complies with the Harmonized USP/EP/JP [1 2 3].

4. Principle

Nitrogen is supplied by the gelatine peptone whilst lactose serves as the fermentable carbohydrate source. The inclusion of bromocresol purple indicator makes the colour change caused by acid production from the fermentation of lactose easy to read with gas formation. The presence of ox bile helps to suppress the growth of Gram-positive and non-enteric bacterial species.

5. Preparation Instructions

Suspend 35.0g/L of dried culture media in deionised/purified water and allow to soak whilst mixing for 10 minutes.
Dispense into suitable containers with Durham tubes added. Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Purple liquid
pH	N/A	7.3 ± 0.2

7. Materials Provided

KM0125 can be provided 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
KM0125-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0125-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0125-10KG-10000	1 x 10kg Dehydrated Culture Media 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.

9. Specimens

KM0125 MacConkey Broth (USP) is suitable for the testing of the following specimens:

- 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

Allow media to come to room temperature.

Food/Environmental Specimens [1 2 3]

- Process the food/environmental specimen as required by local guidelines.
- Solid samples: Prepare a homogenised suspension using a ratio of 1:9 of sample to a non-selective broth such as Tryptone Soya Broth (E&O BM0432) and incubate at 30-35°C aerobically for 18-24 hours.
- After incubation, prepared KM0125 medium should be inoculated with 1ml of the specimen using a sterile pipette. For the testing of gas production, once inoculated, add a Durham tube to the broth vial. If a Durham tube is not used, overlay the inoculated MacConkey Broth (USP) with a plug of petroleum jelly. Cover the broth layer entirely without introducing air.
- Incubate at 42-44°C aerobically for 24-48 hours.
- Presumptive coliforms/*E. coli* should be subcultured onto a poured plate medium, such as MacConkey Agar (E&O PP0640) for further confirmatory tests.

After incubation, examine broths for colour change from purple to yellow, indicating positive lactose fermentation (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
E. coli (NCTC 12923)	43 ± 1°C aerobically for 24-48 hours	Broth: Turbid and yellow. Subplate: Red colonies
S. typhimurium (NCTC 12023)	43 ± 1°C aerobically for 24-48 hours Subcultured onto E&O PP0470 MacConkey without Salt Agar and incubated at 30-35°C aerobically for 18-72 hours.	Broth: Turbid and purple. Subplate: Yellow colonies
S. aureus (NCTC 10788)	43 ± 1°C aerobically for 48 hours Subcultured onto E&O PP0280 Tryptone Soya Agar and incubated at 37 ± 1°C aerobically for 18-24 hours.	Inhibited

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 KM0125 MacConkey Broth (USP) performance, dehydrated culture media samples were used to prepare MacConkey Broth (USP) and tested for turbidity, as well as colony morphology when 1µl on incubated culture in MacConkey Broth (USP) was subcultured onto E&O PP0470 MacConkey without Salt. Prepared broth samples were incubated at 43 ± 1°C aerobically for 24-48 hours, and PP0470 and PP0280 subcultures were incubated at 30-35˚C aerobically for 18-72 hours and 37 ± 1˚C for 18-24 hours respectively. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Escherichia coli* (NCTC 12923) and *Salmonella typhimurium* (NCTC 12023) and no recovery of the non-target test organism: *Staphylococcus aureus* (NCTC 10788). Therefore, it can be concluded that KM0125 MacConkey Broth (USP), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Biochemical and, if indicated, serological testing using pure cultures may be necessary for complete identification; these tests should be performed from a non-selective agar.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH 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 KM0125 Material Safety Data Sheet.

15. Storage conditions and Shelf life

Store product 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 the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the

expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. United States Pharmacopeial Convention (2019) The United States Pharmacopeia USP42, The National Formulary NF37. Rockville, MD: United States Pharmacopeial Convention.
2. Council of Europe (2019) European Pharmacopoeia. 10th ed., Strasbourg: Council of Europe.
3. The Ministry of Health, Labour and Welfare (2021) The Japanese Pharmacopoeia. 18th ed. Tokyo: Yakuji Nippo Limited.

Version History*

001 05/10/23 - New Document Created

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

EC REP

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TABLE OF APPLICABLE SYMBOLS

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