

Instructions for Use

REF - KM0172

Urea Agar Base

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0172 Urea Agar Base, when supplemented with Urea 40% Solution for the preparation of Christensen's medium ⁽¹⁾, is used to detect urease activity in Enterobacteriaceae, particularly *Proteus* spp., isolated from a variety of specimens.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Peptone	1.0
Glucose	1.0
Potassium dihydrogen phosphate	2.0
Sodium chloride	5.0
Phenol red	0.012
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Urea Agar is for the detection of rapid urease activity in Enterobacteriaceae and is prepared according to Christensen's ⁽¹⁾ modification of the Rustigian and Stuart ⁽²⁾ formula. The medium may be used for the detection of urea hydrolysis by some other Enterobacteriaceae, but the incubation period is much longer at 24-48 hours.

Urea Agar is recommended by ISO 6579-1:2017 ⁽³⁾, ISO 10273:2017 ⁽⁴⁾, FDA BAM ⁽⁵⁾ and the UK Standards for Microbiology Investigations ⁽⁶⁾ for the detection of urease activity.

4. Principle

Urea Agar Base is a lightly buffered medium containing urea and phenol red, as the pH indicator. When organisms utilise urea, ammonia is formed which generates an alkaline pH in the medium. As a result of this pH shift, the phenol red changes the medium colour from pale-orange to pink-red, indicating positive urease activity. Gelatine peptone promotes rapid growth of enteric Gram-negative bacilli, permitting a decrease in incubation time. Glucose stimulates urease activity in those organisms which hydrolyse urea slowly.

5. Preparation Instructions

Suspend 2.1g in 95ml of deionised/purified water. Mix with frequent agitation for 10 minutes to completely dissolve the medium. Sterilise at 115°C for 10 minutes before cooling to 45-50°C.

Aseptically add 5ml of sterile 40% urea solution.

Gently mix the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Pale orange firm gel
pH	N/A	6.8 ± 0.2

7. Materials Provided

KM0172 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
KM0172-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0172-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0172-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.*, autoclave, sterile loops, inoculating needle, petri dishes, collection containers, incubators, and quality control organisms.

9. Specimens

KM0172 Urea Agar Base, when supplemented with Urea 40% Solution for the preparation of Christensen's medium ⁽¹⁾, is suitable for the testing of the following specimens:

- Specimens should be individual isolated colonies of interest, or stock cultures.

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 the plates or slopes to come to room temperature.

Isolated Colonies or Stock Cultures:

Inoculate colony directly with an isolated colony from a pure culture by streaking the surface of the plate or slope (do not stab inoculate the butt of the slope, as it can serve as a colour control, for the rate of urease activity)

Slopes: Inoculated media should be incubated aerobically, with the cap loosened at 35-37°C for up to 24 hours.

Urea Agar may be examined for colour change after 2-6 hours of incubation:

- For rapid urease positive organisms (e.g. *Proteus* spp.), a strong positive reaction shown by a deep pink colour throughout the medium, should be visible after 6 hours incubation.
- For delayed positive organisms (*Enterobacter* or *Klebsiella*), a weak reaction may be observed after 6 hours, and the pink colour will intensify over a longer incubation period.
- For suspected yeasts, *Bordetella* and *Brucella* species, the inoculated medium should be incubated for up to 6 days before being considered negative.

Any sign of a pink colour is considered a positive result for urease activity. 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)
<i>E. coli</i> (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: No colour change
<i>P. mirabilis</i> (NCTC 10975)	37 ± 1°C aerobically for 18-24 hours	Growth: Medium turns a deep pink colour

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 KM0172 Urea Agar Base performance, dehydrated culture media samples were used to prepare E&O BM0450 Urea Agar Slope and tested to assess colour change and recovery level when incubated at 37 ± 1°C aerobically for 18-24 hours. All samples of prepared media produced grew and showed the correct colour change of the target organisms: *Escherichia coli* (NCTC 12241), and *Proteus mirabilis* (NCTC 10975). Therefore, it can be concluded that KM0172 Urea Agar Base, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations

- 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 on this medium.
- Extended incubation periods (48+ hours) may produce false-positive results due to metabolism of amino acids. For example, *Pseudomonas aeruginosa* may generate an alkaline pH due to peptone utilisation.
- Heat may decompose urea reducing the efficacy of the medium if the base medium is not cooled sufficiently prior to addition of the urea.
- Liquid cultures should not be used to inoculate Urea agar.

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 KM0172 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 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. Christensen, W. (1946). Urea Decomposition as a Means of Differentiating Proteus and Paracolon Cultures from Each Other and from Salmonella and Shigella Types. J. Bacteriol., 52(4), 461-466.
2. Stuart, C. , Van Stratum, E., and Rustigian, R. (1945) Further Studies on Urease Production by Proteus and Related Organisms. J Bacteriol, 49(5), 437–444.
3. International Organization for Standardization (2017) 6579-1:2017 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella. Geneva: ISO.
4. International Organization for Standardization (2017) 10273:2017 Microbiology of the food chain - Horizontal method for the detection of pathogenic *Yersinia enterocolitica*. Geneva: ISO.
5. FDA Bacteriological Analytical Manual (2005) Chapter 5: Salmonella, 18th Ed., AOAC, Washington, DC.
6. Public Health England (2025) Urease test. UK Standards for Microbiology Investigations. Bacteriology – Test Procedures, TP 36(4.1). London: Standards Unit, Microbiology Services.

Version History*

- 001 26/10/2022 - New Document Created
- 002 20/08/2026 - Updated summary, formats, sample types, methodology, QC testing procedure, limitations and references to harmonise with device file and other, similar products.

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



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TABLE OF APPLICABLE SYMBOLS					IFU/KM0172 REV. 002	
 REF Catalogue number	 LOT Batch code	 IVD In vitro Diagnostic Medical Device	 Manufacturer	 Use by		
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place		