Instructions for Use

REF - KM0082 **R2A Agar**

Dehydrated Culture Media

1. Intended Use

KM0082 R2A Agar is used for the recovery and enumeration of bacteria from drinking water. It is a nutritionally restricted medium that in combination with reduced incubation temperatures and increase incubation times, provides enhanced recovery of stressed and chlorine damaged organisms from treated waters resulting in more realistic bacterial counts.

2. Composition*

Ingredient	g/L
Enzymatic digest of casein	0.25
Enzymatic digest of animal tissue	0.25
Acid hydrolysate of casein	0.5
Yeast extract	0.5
Glucose	0.5
Starch	0.5
Dipotassium hydrogen phosphate	0.3
Magnesium sulphate	0.05
Sodium pyruvate	0.3
Agar	15.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

R2A agar developed by Reasoner and Geldreich[1] is a low nutrient medium that can be used in pour plate, spread plate, and membrane filtration methods for heterotrophic plate counts. In combination with a lower incubation temperature and longer incubation period, R2A agar enhances the growth of stressed and chlorine damaged bacteria. Traditionally, nutritionally rich media have been used for this purpose, but these media support the growth of fast-growing bacteria and may suppress slow growing or stressed bacteria found in treated water.

R2A Agar is recommended by the American Public Health Association (APHA) and referenced by the European Pharmacopeia for the testing of potable water [2 3]. R2A Agar is also recommended by the Standing Committee of Analysts [4] for water samples damaged by disinfectants.

4. Principle

Enzymatic digest of casein, proteose peptone, acid hydrolysate of casein and yeast extract supply the required nitrogen and other essential nutrients. Glucose is a fermentable carbohydrate that provides carbon and energy. Dipotassium hydrogen phosphate is a buffering agent to stabilise pH and magnesium sulphate is present to supply magnesium ions. Starch and sodium pyruvate are added to improve the recovery of stressed organisms.

5. Preparation Instructions

Suspend 18.15g/L of dried culture media in deionised/purified water and mix for 10 minutes.
Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.
Aseptically dispense the specified volume to appropriate sterile containers and allow to cool.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Opalescent firm gel
pH	N/A	7.2 ± 0.2

7. Materials Provided

KM0082 is available in the formats detailed below. Each tab is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

Product Code	Product Format
KM0082-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0082-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0082-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms.

9. Specimens

KM0082 R2A Agar is suitable for the testing of the following specimens:

- Various types of waters, especially high purity waters, beers, and bottled waters.

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

Water Specimens [4]

Based on sample type and information provided, determine whether the specimen requires pre-enrichment or dilution prior to inoculation to the prepared medium. R2A Agar may be used in poured plate, spread plate and membrane filtration procedures [2 4]. Follow local, national or international standards or guidelines for the specific sample type or test required.

- Process specimen as required by selected guidelines.
- For poured plate method: Inoculate sample directly into an empty Petri dish and overlay with medium prepared from KM0082 (Section 5), ensuring the sample and medium are gently mixed.
- For spread plate method: Dispense the molten medium prepared from KM0082 into an empty Petri dish and allow to solidify. Inoculate sample directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.
- For filtration method: filter the required volume of water through a ≤0.45µm filter and place the filter directly onto the agar surface avoiding bubble or inclusions.
- Incubate aerobically at 22°C for 5-7 days and concurrently at 30°C for 3 days or as required by the standard/guidelines being followed.

The European Pharmacopeia recommends incubation at 30-35 °C for ≤3 days [3].

After incubation, examine agar for colonies (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)
A. hydrophila (NCTC 8049)	37 ± 1°C aerobically for 48-72 hours	Grey colonies
P. aeruginosa (NCTC 12903)	37 ± 1°C aerobically for 48-72 hours	Grey colonies with green pigmentation
S. aureus (ATCC 6538)	37 ± 1°C aerobically for 48-72 hours	Grey colonies
E. faecalis (NCTC 12697)	37 ± 1°C aerobically for 48-72 hours	Grey colonies
E. coli (NCTC 12241)	37 ± 1°C aerobically for 48-72 hours	Cream colonies

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 KM0082 R2A Agar performance, dehydrated culture media samples were used to prepare R2A Agar and tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a reference medium. Samples were inoculated with 30-150cfu and incubated aerobically at 37 ± 1°C for 48-72 hours. All samples grew and showed good recovery and the correct morphology of the required test organisms: *Aeromonas hydrophila* (NCTC 8049), *Pseudomonas aeruginosa* (NCTC 12903), *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (NCTC 12697) and *Escherichia coli* (NCTC 12241). Therefore, it can be concluded that KM0082 R2A Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Due to natural variation, some strains may grow poorly on this medium.
- A longer incubation may be necessary for good recovery of slow-growing bacteria.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to KM0082 Material Safety Data Sheet. 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.

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, has passed the expiry date, or the packaging has been damaged.

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. Reasoner, D., and Geldreich, E. (1979) A new medium for the enumeration and subculture of bacteria from potable water. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.
2. APHA (2017) Standard Methods for the Examination of Water and Wastewater, 23rd Edition. Washington DC: APHA
3. Council of Europe (2019) "Water for Injections (Aqua ad iniectabilia)". European Pharmacopoeia. 10th ed., Strasbourg: Council of Europe.
4. The Microbiology of Drinking Water (2020) Part 7 – Methods for the enumeration of heterotrophic bacteria. Standing Committee of Analysts, Environment Agency, Nottingham, UK.

Version History*

001 12/09/23 - New Document Created

*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