Instructions for Use

REF - LS0054 Legionella BCYE without Cysteine Supplement

Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use. LS0054 Legionella BCYE without Cysteine Supplement is a lyophilised selective supplement used to produce Legionella BCYE Cysteine Free Agar. This agar is intended for use as a secondary diagnostic medium for confirmation of a previously isolated organism in conjunction with Legionella BCYE Agar (containing cysteine). Although it contains ferric pyrophosphate and α-ketoglutarate it does not contain L-cysteine so will not support the growth of *Legionella* spp.

2. Composition*

For one litre of prepared media

Ingredient	g/L
α-Ketoglutaric acid	1.0
ACES buffer	10.0
Potassium hydroxide	2.8
Iron (III) pyrophosphate	0.25

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Legionella BCYE without Cysteine is intended for use in conjunction with Legionella BCYE Agar (with cysteine) as a secondary diagnostic medium for confirmation of a previously isolated organism. Although it contains ferric pyrophosphate and α-ketoglutarate it does not contain L-cysteine so will not support the growth of *Legionella* spp.

Legionella spp. are aerobic, motile, nutritionally fastidious, Gram-negative rods that require specific growth factors, including L-cysteine, in culture media to proliferate [1,2]. Transmission mostly occurs through aerosolization or aspiration of contaminated water. Legionellosis refers to two distinct clinical syndromes, Legionnaires' disease, which most often presents as severe pneumonia, and Pontiac fever, which is an acute, febrile, self-limiting, viral-like illness. Over fifty species of *Legionella* have been identified, however *Legionella pneumophila* remains the most prevalent causative agent in Legionnaires' disease. *Legionella* spp. may be isolated from respiratory tract secretions of all types, sterile body fluids such as blood, and biopsy material.

LS0054 Legionella BCYE without Cysteine Supplement is designed to be used with E&O KM0015 Legionella Agar Base to form Legionella BCYE Cysteine Free Agar. When used in the final medium, LS0054 is as recommended by ISO 11731:2017 [3] and UK Standards for Microbiology Investigations [4] and is tested in accordance with ISO 11133:2014 [5].

Cysteine is an absolute requirement for growth of *Legionella* spp. Gram negative organisms that grow on Legionella BCYE Agar (with cysteine) but fail to grow on Legionella BCYE Cysteine Free Agar, may be presumptively identified as *Legionella* spp. Further testing may be required (e.g. biochemical, immunological, molecular, or mass spectrometry techniques) for definitive identification.

4. Principle

Legionella BCYE without Cysteine Supplement contains α-ketoglutarate as a growth stimulant, ACES buffer to control pH, and ferric pyrophosphate as a source of essential Fe^{3+} ions [6]. No cysteine is present so the medium produced is deficient in this essential factor for the growth of *Legionella* spp.

5. Preparation Instructions

To reconstitute the contents of each vial, aseptically add the recommended volume of sterile deionised water:

Format	Volume of sterile deionised water to add
V003	50ml

Close the vial and gently agitate to assist reconstitution – avoid frothing. The resulting solution will be free from visible particulate matter.

Add the contents of the vial to the appropriate volume of sterile base medium (such as KM0015 Legionella Agar Base) prepared according to the instructions provided and allowed to cool to 45-50°C. Mix gently but thoroughly and pour aseptically into sterile Petri dishes.

6. Physical Characteristics

	Pellet Appearance	Reconstituted
Appearance and Colour	Beige Solid Pellet	Straw liquid

7. Materials Provided

LS0054 Legionella BCYE without Cysteine Supplement is supplied in boxes of 10 vials (lyophilised). Product code: LS0054-V003-500. Each vial is labelled with (abbreviated) product name, product code, lot number, expiry date and the required volume of prepared medium.

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile deionised water for reconstitution, autoclave, sterile loops or swabs, incubators, quality control organisms, and dehydrated culture medium KM0015 Legionella Agar made up according to instructions.

9. Specimens

LS0054 Legionella BCYE without Cysteine Supplement is designed to be used with KM0015 Legionella Agar Base to form Legionella BCYE Cystine Free Agar, which is suitable for the testing of the following specimens:

- Water Samples: Potable waters, water from heating systems, cooling towers and air conditioning systems.
- Clinical: Suspected *Legionella* spp. from respiratory tract secretions of all types, blood, and biopsy material.

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/Environmental Specimens [3]

Legionella BCYE Cysteine Free Agar (produced using LS0054) is recommended for the confirmation of presumptive *Legionella* spp. colonies found on other selective Legionella media. Subculture presumptive or suspected colonies from the primary isolation medium to both Legionella BCYE without Cysteine Agar and Legionella BCYE Agar (with cysteine). Incubate at 36 ± 2°C in 5% CO_2 and high humidity for 2 to 5 days. Subcultured colonies that grow on Legionella BCYE Agar but not on Legionella BCYE without Cysteine Agar may be regarded as *Legionella* spp.

According to ISO 11731 [3], the method required for enumerating *Legionella* spp. is determined by the assumed sample content. Non-selective media such as Legionella BCYE Agar are recommended when processing samples that are presumed to have higher numbers of *Legionella* species in combination with lower numbers of interfering microorganisms. In contrast, selective media such as Legionella GVPC Agar are recommended when processing samples that are presumed to have lower concentrations of *Legionella* species in combination with higher numbers of interfering microorganisms. These samples are processed as required by local guidelines.

Presumptive *Legionella* spp. will produce colonies on Legionella BCYE Agar but not on Legionella BCYE Cysteine Free Agar.

Clinical Samples [4]

UKHSA Standards for Microbiology Investigations ID 18 i3 [4] recommends the use of Legionella BCYE Agar (with cysteine) and Legionella BMPA Agar as primary isolation media and the use of Legionella BCYE Cysteine Free Agar as part of the confirmation of the presence of *Legionella* spp. Follow local, national, or international guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
S. aureus (NCTC 12981)	37 ± 1°C in 5% CO_2 for 3-5 days	White colonies
L. pneumophila (NCTC 11192)	37 ± 1°C in 5% CO_2 for 3-5 days	Inhibited

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

12. Performance

To fully verify LS0054 Legionella BCYE without Cysteine Supplement performance, samples were used to prepare Legionella BCYE Cysteine Free Agar and resulting plates tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10^4 -10^5 cfu for the non-target organisms and incubated at 37 ± 1°C in 5% CO_2 for 3-5 days. All samples showed expected recovery and morphology of *Staphylococcus aureus* (NCTC 12981), and no recovery of Legionella *pneumophila* (NCTC 11192). Therefore, it can be concluded that LS0054 Legionella BCYE without Cysteine Supplement meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Due to natural variation, some strains may grow poorly on this medium.
- 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.
- Not all *Legionella*-positive samples may be identified by a single culture method. A combination of non-selective and selective media is strongly recommended.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to LS0054 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.

Do not use if supplement is not specified colour, has been stored inappropriately or the packaging has been damaged. Any unused supplement should be discarded.

15. Storage conditions and Shelf life

Legionella BCYE without Cysteine Supplement (LS0054) must be stored at 2-8°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements.

16. References

1. Feeley, J., Gibson, R., Gorman, G., Langford, N., Rasheed, J., Mackel, D., and Baine, W. (1979) Charcoal-yeast extract agar: primary isolation medium for *Legionella pneumophila*. Journal Of Clinical Microbiology, 10(4), pp.437-441.
2. Fields, B., Benson, R., and Besser, R. (2002) *Legionella* and Legionnaires' Disease: 25 Years of Investigation. Clinical Microbiology Reviews, 15(3): 506-526.
3. International Organization for Standardization (2017) 11731:2017 Water quality - Enumeration of *Legionella*. Geneva, ISO.
4. Public Health England (2015) Identification of *Legionella* species. UK Standards for Microbiology Investigations. Bacteriology, SMI 18(3). London: Standards Unit, Microbiology Services.
5. 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.
6. Edelstein, P. (1983) Comparative Study of Selective Media for Isolation of *Legionella pneumophila* from Potable Water. Journal Of Clinical Microbiology, 18(3): 751-751.

Version History*

001 23/08/2016 - Document created.
002 05/07/2023 - Fully updated IFU to new standard.
003 05/02/2024 - Updated to state "hazardous" in Section 13 after review of new standard.
004 13/10/2025 - Update to Section 4 to include reconstitution volumes.

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

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TABLE OF APPLICABLE SYMBOLS

REF o 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