INSTRUCTIONS FOR USE

LEGIONELLA BCYE α-GROWTH SUPPLEMENT W/O CYSTEINE

Freeze-dried enrichment supplement

1 - INTENDED USE

In vitro diagnostic. Mixture of growth factors lacking L-cysteine to be used with Legionella BCYE Agar Base for the confirmation of *Legionella* colonies isolated from clinical specimens and water samples.

2 - COMPOSITIONS - (VIAL CONTENTS FOR 500 ML OF MEDIUM)

ACES Buffer/Potassium hydroxide	6.4 g
α–ketoglutarate monopotassium salt	0.5 g
Ferric pyrophosphate	125.0 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Legionella BCYE α-Growth Supplement w/o Cysteine is a freeze-dried mixture of growth factors, lacking L-cysteine, to be used as a supplement to BCYE Agar Base (REF 401582) for the for the confirmation of *Legionella* colonies isolated from clinical specimens and water samples.
ACES Buffer is used for pH stabilisation, α-ketoglutarate and ferric pyrophosphate stimulate *Legionella* growth. L-cysteine, is an essential amino acid and an important energy source for *Legionella* spp. and is not included in the supplement: the differentiation of *Legionella* colonies is obtained by their inability to grow on the medium without L-cysteine.

4- DIRECTIONS FOR MEDIA PREPARATION

Reconstitute the contents of one vial of BCYE α-Growth Supplement w/o Cysteine with 50 mL of sterile purified water.
Add to 450 mL of Legionella BCYE Agar Base (REF 401582) autoclaved at 121°C for 15 minutes and cooled to 47-50°C with aseptic precautions. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Freeze-dried supplement appearance	medium size, pink pastille
Aspect of the solution	light yellow, opalescent

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Legionella BCYE α-Growth Supplement w/o Cysteine	Freeze-dried supplement	423212	4 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Legionella BCYE Agar Base (REF 401582), autoclave, incubator and laboratory equipment as required, autoclavable flasks, sterile loops and swabs, reagents for the sample treatment, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Legionella BCYE Agar Base supplemented with Legionella BCYE α-Growth Supplement w/o Cysteine, must be inoculated with colonies cultivated on selective or non-selective isolation media for the presumptive confirmation of *Legionella* colonies.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
A first criterion to differentiate *Legionella* colonies is their inability to grow, with rare exceptions (*L.oakridgensis*, *L.jordanis*, and *L.nagasakiensis, L.spiritensis*)[1,2,3], on medium lacking L-cysteine.
When there is only one colony type, pick three presumptive colonies; if more morphological different types of presumptive colonies of *Legionella* are growing on the plate, take at least one colony from each type.[2]
Subculture onto a plate of BCYE with cysteine and a plate of BCYE without cysteine.
Be careful not to carry over any culture media with the colony and first inoculate a plate of Legionella Agar without cysteine.
Incubate at 36 ± 2°C for 2 to 5 days.[2]

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth on both inoculated plates. Regard as *Legionella* those colonies which grow on the plate of BCYE with cysteine but fail to grow on the plate of BCYE without cysteine.
Presumptive identification should be completed by Gram staining prepared from cysteine containing agar only: *Legionella* cells are Gram-negative poorly/faintly staining thin rods, which may be filamentous in older cultures.[2]

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Consult the quoted literature for the details of the quality control procedures.[2,4,5]

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12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of Legionella BCYE α-Growth Supplement w/o Cysteine used for the supplementation of dehydrated Legionella Agar Base REF 401582 is tested for productivity/selectivity properties comparing the results with a previously approved batch.
The property of the medium not to allow growth of *Legionella* spp. is tested by inoculating the following strains: *L.pneumophila* ATCC 33152, *L.pneumophila,* clinical isolate and *L.anisa* ATCC 35292. The property of the medium to allow the growth of non-*Legionella* strains is tested with *E.coli* ATCC 25922 and *S.aureus* ATCC 25923. After incubation at 35-37°C for 48-72 hours non-*Legionella* strains show a good growth while *Legionella* strains do not growth.

13 - LIMITATIONS OF THE METHOD

- The plates with characteristic growth and with colonies presumptively identified as *Legionella*, must undergo confirmation tests with biochemical, immunological, molecular or mass spectrometry techniques. If relevant, perform antimicrobial susceptibility testing.
- In clinical microbiology, the diagnosis of legionellosis must be based on an interdisciplinary approach that includes radiological results, cultural results, determination of urinary antigen. BCYE supplement and the medium base are intended as an aid to the diagnosis of the infection: the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of the microscopic and/or other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- BCYE Supplement w/o Cysteine is a qualitative *in vitro* diagnostic, for professional use only; it must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- BCYE Supplement w/o Cysteine is classified as dangerous according to current European legislation; consult the Safety Data Sheet before use.
- The supplement and the medium base shall be used in association according to the directions described above. Apply Good Manufacturing Practice in the production process of prepared media.
- BCYE Supplement w/o Cysteine is sterilized by membrane filtration.
- Be careful when opening the metal ring to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplements or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused supplements and the sterilized media inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use BCYE Supplement w/o Cysteine as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

1. Mercante JW, Winchell JM. Current and Emerging Legionella Diagnostics for Laboratory and Outbreak Investigations. Clin Microbiol Rev. 2015; 28:95-147
2. ISO 11731:2017 Water quality — Enumeration of Legionella
3. Public Health England. UK Standards for Microbiology Investigations. Identification of Legionella species. ID18, Issue no: 3, Issue date: 14.04.15
4. Feeley JC, Gibson RJ, Gorman GW, Langford NC, Rasheed JK, Mackel DC, Baine WB, Charcoal-yeast extract agar: primary isolation medium for Legionella pneumophila, J Clin Microbiol 1979; 10:437-441.
5. Edelstein P.H., Improved semiselective medium for isolation of Legionella pneumophila from contaminated clinical and environmental specimens. J Clin Microbiol 1981; 14:298-303

423212 LEGIONELLA BCYE α-GROWTH SUPPLEMENT W/O CYSTEINE
SDS
Regulation (EU) 2020/878

Contains: POTASSIUM HYDROXIDE

Classification

Substance or mixture corrosive to metals, category 1	H290	May be corrosive to metals.
Acute toxicity, category 4	H302	Harmful if swallowed.
Skin corrosion, category 1A	H314	Causes severe skin burns and eye damage.
Serious eye damage, category 1	H318	Causes serious eye damage.

Labelling
Pictogram

Signal word	Warning
Hazard statement(s)	
H290	May be corrosive to metals.
H302	Harmful if swallowed.
H314	Causes severe skin burns and eye damage.
Precautionary statements:	
P260	Do not breathe dust / fume / gas / mist / vapours / spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P310	Immediately call a POISON CENTER / doctor / . .
P264	Wash thoroughly after handling.

TABLE OF APPLICABLE SYMBOLS

REF or 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	Store away from direct light	Fragile, handle with care

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/01
Revision 3	Removal of obsolete classification	2023/04

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