INSTRUCTIONS FOR USE

LEGIONELLA BCYE α-GROWTH SUPPLEMENT

Freeze-dried enrichment supplement

1 - INTENDED USE

In vitro diagnostic. Mixture of growth factors to be used with Legionella BCYE Agar Base for the isolation and enumeration of *Legionella* spp. from clinical specimens and water samples.

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

ACES Buffer/Potassium hydroxide	6.4 g
Alpha-ketoglutarate, monopotassium salt	0.5 g
Ferric pyrophosphate	125 mg
L-Cysteine HCl	200 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Legionella BCYE α-Growth Supplement is a freeze-dried mixture of growth factors to be used as a supplement to BCYE Agar Base (REF 401582) for the isolation and enumeration of *Legionella* spp. in clinical specimens and in waters.
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

4- DIRECTIONS FOR MEDIA PREPARATION

Reconstitute the contents of one vial of Legionella BCYE α-Growth Supplement with 50 mL of sterile purified water.

NON-SELECTIVE MEDIUM WITH CYSTEINE: BCYE W/ L-CYSTEINE

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.

Selective medium

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. Add also the contents of one vial of the suitable selective supplement (e.g., GVPC REF 423210, or MWY REF423220 or AB REF 423225). 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	Freeze-dried supplement	423210	4 vials, each for 500 mL of medium CND W0104010104; EDMA: 14.01.01.04; RDM1892731/R

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

The complete media are intended for the bacteriological processing of several human clinical specimens.[1,2] and all kinds of water samples.[4] Good laboratory practices for collection, transport and storage of the specimens should be applied.

9 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.

Isolation from clinical specimens[1,2]

Inoculate approximately 0.1 mL of treated specimen onto each plate, with the bulk of inoculum applied to the first quadrant and streak with a loop over the other quadrants of the plate to obtain well isolated colonies.
Incubate at 35-37°C in humidified air for 14 days. Colonies are normally microscopically visible after 2 days and, macroscopically, after 3-5 days. For operational details, consult the cited bibliography and the instructions for use of the dehydrated medium (REF 401582).

Enumeration in environmental samples[3]

The work procedures described in the ISO 11731 Standard differ in relation to the origin of the sample, its characteristics, the purposes of the research and in relation to the expected concentrations of the target microorganism and the contaminating flora.
For operational details, consult the cited bibliography and the instructions for use of the dehydrated medium (REF 401582).

10 - READING AND INTERPRETATION

Isolation and enumeration

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristic of the colonies.
For details, consult the cited bibliography and the instructions for use of the dehydrated medium (REF 401582).

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, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of *Legionella* strains and

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: export@biolifeitaliana.it; web: www.biolifeitaliana.it

non-target microorganisms must be made depending on of the prepared, selective or non-selective, media and the field of application (clinical or water analysis). Consult the quoted literature for the details of the quality control procedures.[4,5,6]

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of BCYE α-Growth Supplement (REF 423210) used for the supplementation of dehydrated Legionella Agar Base REF 401582 (Test Batch-TB) is tested for productivity comparing the results with a previously approved batch (Reference Batch-RB)

Productivity is tested by a quantitative method, with the following strains: *L.pneumophila* ATCC 33152, *L.pneumophila,* clinical isolate and *L.anisa* ATCC 35292. Test Batch and Reference Batch are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 35-37°C for 44-48 hours (*L.pneumophila)* and 3-5 days *(L.anisa)*. The colonies are enumerated on both batches and the productivity ratio (Pr= CFU_{TB}/CFU_{RB}) is calculated. If *Pr* is ≥ 0,7 and if the colonies morphology is typical, the results are considered acceptable and conform to the specifications.

13 - LIMITATIONS OF THE METHOD

- Some legionellae cannot be grown on routine Legionella culture media and have been termed Legionella-like amoebal pathogens (LLAPs), because they grow in certain host species of amoeba.[7]
- Colonies of *Legionella* grown on white membrane filters may have a different appearance to those that develop against a black or dark background filter.
- Do not incubate the medium with CO_2 concentrations above 2.5% as growth of L.pneumophila may be inhibited.[8]
- The glycine contained in the medium may inhibit some of non-*pneumophila* strains.[9]
- Selective BCYE media that contain vancomycin may not support the growth of all *Legionella* spp.[10]
- Not all *Legionella*-positive samples may be identified by a single culture method. A combination of non selective and selective media is strongly recommended. [1,2,11]
- 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 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 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 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 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

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2. Public Health England. UK Standards for Microbiology Investigations. Identification of Legionella species. ID18, Issue no: 3, Issue date: 14.04.15 Microbiology; 2015.
3. ISO 11731:2017 Water quality — Enumeration of Legionella
4. ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media
5. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004.
6. The Australian Society for Microbiology. Guidelines for Assuring Quality of Medical Mycological Culture Media. 2012
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8. 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
9. Lück PC, Igel L, Helbig JH, Kuhlisch E, Jatzwauk L. Comparison of commercially available media for the recovery of Legionella species. Int J Hyg Environ Healt 2004; 207(6):589-93

10. Lee TC, Vickers RM, Yu VL, Wagener MM. Growth of 28 Legionella species on selective culture media: a comparative study. J Clin Microbiol 1993;31(10):2764-
11. Kusnetsov JM, Jousimies-Somer HR, Nevalainen AI, Martikainen PJ. Isolation of Legionella from water samples using various culture methods. J Appl Bacteriol. 1994 76(2):155-62.

423210 LEGIONELLA BCYE α-GROWTH SUPPLEMENT
SDS rev 2
Regulation (EU) 2020/878

Mixtures containing potassium hydroxide

Classification

Substance or mixture corrosive to metals, category 1	H290	May be corrosive to metals.
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.
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	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/01

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