INSTRUCTIONS FOR USE

LEGIONELLA BCYE AGAR BASE

LEGIONELLA BCYE α-GROWTH SUPPLEMENT
LEGIONELLA BCYE α-GROWTH SUPPLEMENT W/O CYSTEINE

LEGIONELLA GVPC SELECTIVE SUPPLEMENT
LEGIONELLA AB SELECTIVE SUPPLEMENT
LEGIONELLA MWY SELECTIVE SUPPLEMENT (ISO)

Dehydrated culture medium and supplements

Legionella Agar (BCYE): colonies of *L. pneumophila* and of contaminating flora

1 - INTENDED USE

In vitro diagnostics. Medium base and growth and selective supplements for the isolation and enumeration of *Legionella* spp. from clinical specimens and water samples.

2 - COMPOSITIONS

LEGIONELLA BCYE AGAR BASE
TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Activated charcoal	2.0 g
Yeast extract	10.0 g
Agar	13.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

LEGIONELLA BCYE α-GROWTH SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

ACES Buffer/Potassium hydroxide	6.4 g
α–ketoglutarate	0.5 g
Ferric pyrophosphate	125.0 mg
L-Cysteine HCl	200.0 mg

LEGIONELLA BCYE α-GROWTH SUPPLEMENT W/O CYSTEINE
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

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

LEGIONELLA GVPC SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Glycine	1.5 g
Vancomycin HCl	0.5 mg
Polymyxin B	40.000 IU
Cycloheximide	40.0 mg

LEGIONELLA AB SELECTIVE SUPPLEMENT
(VIAL CONTENTS FOR 500 mL OF MEDIUM)

Cefazolin	4.5 mg
Polymyxin B	40,000 UI
Pimaricin (natamycin)	35 mg

LEGIONELLA MWY SELECTIVE SUPPLEMENT (ISO)
(with anisomycin)
(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Glycine	1.5 g
Vancomycin HCl	0.5 mg
Polymyxin B	25.000 UI
Anisomycin	40 mg
Bromothymol blue	5.0 mg
Bromocresol purple	5.0 mg

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Legionellae are mesophilic, motile, a-saccharolytic, obligately aerobic, nutritionally fastidious, Gram-negative, non-spore-forming gammaproteobacteria.[1] *Legionella pneumophila*, the most widely studied species, displays pleomorphism, demonstrating coccoid, bacillary and/or long filamentous forms that are influenced by temperature, available nutrients or metabolites, growth environment and medium type.[2] *Legionella* species share growth dependence for L-cysteine and growth enhancement by iron.[1] Legionellae grow on several types of complex artificial media, however, the most successful medium is buffered charcoal yeast extract (BCYE) agar containing ferric pyrophosphate, α-ketoglutarate and L-cysteine.[2]

The culture is the reference technique for laboratory diagnosis: it has 100% specificity and a variable sensitivity depending on the characteristics of the sample, on the experience and technical proficiency of laboratory personnel, as well as on the delays in respiratory sample processing, the prior use of antimicrobial therapies and culture overgrowth by other oropharyngeal bacteria.[2,3]

Optimal yield of *Legionella* spp. from clinical specimens usually requires that a variety of media be used: one plate with non-selective medium (BCYE) and two with selective media.[1]

The choice of the method used for the enumeration of *Legionella* spp. in water depends on the origin and characteristics of the sample, the reason of sampling or investigation, the expected concentration of interfering microorganisms and the desired lower limit of detection level; a decision matrix for choosing an appropriate method is described in ISO 11731.[4]

Buffered Charcoal Yeast Extract Agar (BCYE) was developed by Feeley et al.[5] and then further modified by Edelstein[6] by introducing α-ketoglutarate, and by Pasculle et al.[7] by the addition of ACES buffer.

Wadowsky and Yee[8] proposed a selective version of BCYE, by introducing in the formulation glycine, vancomycin and polymyxin B, resulting in the formation of GVP medium. Another modification in 1984 by Dennis et al.[9] made the medium even more selective for *Legionella* by the addition of cycloheximide, resulting in GVPC medium.

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Vickers et al.[10] introduced 0.001% of bromocresol purple and bromothymol blue into BCYE agar for the differentiation between members of the family *Legionellaceae*. Edelstein in 1982[11] proposed MWY medium as a modification of the GVP medium of Wadowsky and Yee, including bromothymol blue and bromocresol purple and an antifungal agent.
Legionella BCYE Agar Base (401582), GVPC Supplement (423215), AB Selective Supplement (423225), MWY Selective Supplement ISO (423220), BCYE α-Growth Supplement (423210) and BCYE α-Growth Supplement w/o Cysteine (423212) are prepared according to the formulations recommended by ISO 11773.[4]
Yeast extract is a source of nitrogen, carbon, and vitamins for microbial growth. Activated charcoal removes hydrogen peroxide and other toxic products. 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. Glycine and polymyxin B are inhibitors of Gram-negative bacteria, cefazolin is active against Gram-positive bacteria and some Gram-negative bacteria, vancomycin suppress the growth of Gram-positive bacteria while cycloheximide, natamycin and anisomycin are used as antifungal agents.

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend12.5 g of Legionella BCYE Agar Base in 450 mL of cold purified water. Heat to boiling with agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C. Add the suitable growth and selective supplements. After supplements addition, keeping the medium under stirring, distribute into sterile Petri dishes.

SELECTIVE MEDIUM BCYE-GVPC
To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella GVPC Selective Supplement (REF 423215) reconstituted with 10 mL of sterile purified water.
SELECTIVE MEDIUM BCYE-AB
To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella AB Selective Supplement (REF 423225), reconstituted with 5 mL of sterile purified water.
SELECTIVE MEDIUM BCYE-MWY (WITH ANISOMYCIN)
To the medium base cooled to 47-50°C, add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210) reconstituted with 50 mL of sterile purified water and the contents of one vial of Legionella MWY Selective Supplement (ISO) (REF 423220), reconstituted with 10 mL of sterile purified water.
NON-SELECTIVE MEDIUM WITH CYSTEINE: BCYE W/ L-CYSTEINE
To the medium base cooled to 47-50°C add the contents of one vial of Legionella BCYE α-Growth Supplement (REF 423210), reconstituted with 50 mL of sterile purified water.
NON-SELECTIVE MEDIUM WITHOUT CYSTEINE: BCYE W/O L-CYSTEINE
To the medium base cooled to 47-50°C add the contents of one vial of Legionella BCYE α-Growth Supplement w/o Cysteine (REF 423212), reconstituted with 50 mL of sterile purified water.

5 - PHYSICAL CHARACTERISTICS

Legionella BCYE Agar Base (REF 4015822-4015824)	
Dehydrated medium appearance:	fine grain size, blackish
Prepared plates appearance	black, homogeneously opaque
Final pH at 20-25°C	6.9 ± 0.1
Legionella BCYE α-Growth Supplement (REF 423210)	
Freeze-dried supplement appearance	medium size, pink pastille
Aspect of the solution	light yellow, opalescent
Legionella BCYE α-Growth Supplement w/o Cysteine (REF 423212)	
Freeze-dried supplement appearance	medium size,dark-pink pastille
Aspect of the solution	light yellow, opalescent
Legionella GVPC Selective Supplement (REF 423215)	
Freeze-dried supplement appearance	high size, white pastille
Aspect of the solution	colourless, clear
Legionella AB Selective Supplement (REF 423225),	
Freeze-dried supplement appearance	high size, white pastille
Aspect of the solution	whitish, cloudy
Legionella MWY Selective Supplement (ISO) (REF 423220),	
Freeze-dried supplement appearance	high size, bluish pastille
Aspect of the solution	blue, cloudy

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Legionella BCYE Agar Base	Dehydrated medium	4015822 4015824	500 g (20 L) 5 kg (200 L)
Legionella BCYE α-Growth Supplement	Lyophilized supplement	423210	4 vials, each for 500 mL of medium
Legionella BCYE α-Growth Supplement w/o Cysteine	Lyophilized supplement	423212	4 vials, each for 500 mL of medium
Legionella GVPC Selective Supplement	Lyophilized supplement	423215	4 vials, each for 500 mL of medium
Legionella AB Selective Supplement	Lyophilized supplement	423225	10 vials, each for 500 mL of medium For water microbiological control only
Legionella MWY Selective Supplement (ISO)	Lyophilized supplement	423220	4 vials, each for 500 mL of medium For water microbiological control only

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, reagents for the sample treatment, ancillary culture media and reagents for the identification of the colonies.

8 - SPECIMENS

Legionella BCYE Agar base, Legionella BCYE α-Growth Supplement and the selective supplements GVPC, are intended for the bacteriological processing of several human clinical specimens including those from the lower respiratory tract, such as sputum, pleural fluid, bronchial aspirates, and bronchial alveolar lavage (BAL) fluid; lung tissue and biopsy specimens are also appropriate for attempting culture.[1,12] Collect specimens before antimicrobial therapy where possible. Transfer the sample as soon as possible to the laboratory; use a transport medium if the sample cannot be processed immediately. Legionella BCYE Agar Base, Legionella BCYE α-Growth Supplement and the selective supplements GVPC, MWY-ISO and AB are intended for the bacteriological processing of non-clinical specimens: all kinds of water samples such as potable, industrial, waste, natural waters and water related samples (e.g. biofilms, sediments, etc.).[4] Consult the ISO Standard 11731 for sampling methods and for sample treatment procedures.[4] Good laboratory practices for collection, transport and storage of the specimens should be applied.
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.

Isolation from clinical specimens[1,12]

Optimal yield of *Legionella* spp. from clinical specimens usually requires[1]:

- That specimen be diluted 1:10 in Tryptic Soy Broth or distilled water to reduce inhibition by tissue and serum factors, as well as antibiotics. If the sputum is very dense, it must be re-suspended with 0.2-1 mL of dithiothreitol-based fluidifying.
- That the specimen be pre-treated to reduce contaminating flora. This is done by diluting 1:10 the specimen with a low pH KCl-HCl buffer (pH 2.2) and incubating at room temperature for 4 minutes. An alternative to specimen acidification is heating at 50°C for 30 min.
- That a variety of media be used: one plate with non selective medium (BCYE) and two with selective media.

Inoculate approximately 0.1 mL 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. A small amount of CO_2 (2.5%) supplementation may enhance the growth of some of the more fastidious *Legionella* spp. such as *L.sainthelensi* and *Loakridgensis*. This low level of CO_2 supplementation will not harm the growth of *L.pneumophila*, but CO_2 levels higher than 2.5% may inhibit growth.
Colonies are normally microscopically visible after 2 days and, macroscopically, after 3-5 days.

Enumeration in environmental samples[4]

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.
Schematically, the different possibilities of treatment and inoculation of the samples are summarized below.

1. For samples with a high number of *legionellae* and a low number of contaminants: direct inoculation of the sample on a non-selective BCYE w/L-cysteine medium and on a selective BCYE-AB medium plate.
2. For samples with a low number of legionellae and a low number of contaminants: membrane filtration and positioning of the untreated filter on a BCYE w/ L-cysteine non-selective medium plate, positioning of the filter(s) treated with acids on one or more selective or highly selective medium plates (BCYE-AB or BCYE-GVPC or BCYE-MWY); wash the untreated and acid or heat treated membrane and inoculate 0.1-0.5 mL on a non-selective medium plate and on plates of one or more selective and highly selective media (BCYE-AB or BCYE-GVPC or BCYE-MWY).
3. For samples with a high number of contaminants: inoculate the non-concentrated, concentrated and diluted 1:10 sample; divide each sub-sample into three aliquots: one untreated, one treated with heat and one treated with acids; inoculate 0.1-0.5 mL of each aliquot on a selective medium plate (BCYE-GVPC or BCYE-MWY).
4. For samples with a very high number of contaminants: inoculate the un-concentrated and diluted sample 1:10 and 1: 100 after a pre-treatment with a combination of heat followed by the acid solution. Prepare dilutions with the appropriate diluent after acid treatment. After vortexing, inoculate 0.1-0.5 mL of each aliquot on a selective medium (BCYE-GVPC or BCYE-MWY) plate.

Allow the inoculum to absorb well then incubate the inverted plates in a humid atmosphere at 36 ± 2°C for 7-10 days, observing the plates at days 2, 3, 4, 5 and then at the end of the incubation period.
The procedural elements reported above are entirely schematic. For details of *Legionella* counting techniques in water, refer to the ISO 11731 Standard[4] or other applicable guidelines.

Confirmation of the colonies

A first criterion to differentiate *Legionell*a colonies is their inability to grow, with rare exceptions (*L.oakridgensis*, *L.jordanis*, and *L.nagasakiensis, L.spiritensis*)[2,4,12], 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.[4]
Subculture onto a plate of BCYE w/cysteine and a plate of BCYE w/o cysteine.
Be careful not to carry over any culture media with the colony and first inoculate a plate of Legionella Agar w/o Cysteine.
Incubate at 36 ± 2°C for 2 to 5 days.[4]

10 - READING AND INTERPRETATION

Isolation and enumeration

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristic of the colonies.
Legionella spp. colonies begin to appear on culture plates on day 2 of incubation. It is very unusual for the bacterial colonies to appear on plates after 5 days of incubation. Some very rarely isolated *Legionella* spp. may require up to 14 days of incubation before growth appears; this is an extremely rare event. Regardless, it is reasonable to inspect culture plates on days 2 to 5 ad than again at day 14.[1]
In the first 24-36 hours of incubation the observation of the plate under a low power binocular microscope with incident light illuminating the agar surface at an acute angle may helps in the *Legionella* and contaminants colonies recognition.
Legionella colonies, in principle, appear white-gray, with entire, shiny edges, rounded with a diameter of 1 to 4 mm. Generally, and specially in the first 2 days of incubation, the edge shows a pink or blue-green iridescence while the centre is opalescent gray with an appearance similar to ground glass. Observed under UV lamp (366 nm), some species (*L.anisa, L.bozemanii, Lcherrii, L.dumoffii,*

L.gormanii, L.gratiana, L.parisiensis, L.steigerwaltii and *L.tucsonensis*) show a blue-white auto-fluorescence, others (*L.erythra and L.rubrilucens*) a bright red auto-fluorescence. *L.pneumophila* and common legionellae, normally do not show auto-fluorescence. With the prolongation of the incubation time, the colonies become wider, the centre assumes a creamy white colour and lose much of their iridescence. A common feature of *Legionella* colonies is the difficulty in taking them with the loop from the surface of the agar.
For the details of *Legionella* spp. enumeration in water samples consult the ISO Standard.[4]

Confirmation of the colonies

After incubation, observe the bacterial growth on both inoculated plates. Regard as *Legionella* those colonies which grow on the plate of BCYE w/cysteine but fail to grow on the plate of BCYE w/o 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.[4]

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 in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of *Legionella* strains and 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.[13,14,15]

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated Legionella Agar Base REF 401582 (Test Batch-TB) supplemented with BCYE α-Growth Supplement and Legionella GVPC Selective Supplement is tested for productivity and selectivity. 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 water 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.
The productivity of Legionella BCYE Agar Base REF 401582 is also evaluated with the addition of only BCYE α-Growth Supplement with the target strain *L.pneumophila* ATCC 33152 with the same acceptance criteria described above for the medium also added with GVPC.
Selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the following non-target strains: *S.aureus* ATCC 25923, *E.faecalis* ATCC 19433, *E.coli* ATCC 25922, *P.aeruginosa* ATCC 27853 and *C.albicans* ATCC 18804. After incubation at 35-37°C for 72 hours the growth of non-target strain is observed and recorded: *S.aureus*, *E.faecalis* and *E.coli* are totally inhibited, while *P.aeruginosa* and *C.albicans* are partially inhibited.

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.[16]
- Colonies of *Legionella* grown on white membrane filters may have a different appearance to those that develop against a black or dark background filter.
- Feeley et al.[5] recommend not to incubate the medium in CO_2 higher than 2.5% due to the possibility that *L.pneumophila* growth may be inhibited.
- The glycine contained in the medium may inhibit some of non-*pneumophila* strains.[17]
- Selective BCYE media that contain vancomycin may not support the growth of all *Legionella* spp.[18]
- Culture media performance is a critical factor in the isolation of legionellae from respiratory samples. It has been reported[3] that WY media yielded significantly higher isolation rates than GVPC and BCYE media in regard to performance with samples that harboured low *Legionell*a inocula and high contamination levels.
- Not all *Legionella*-positive samples may be identified by a single culture method. A combination of non selective and selective media is strongly recommended.[1,12,19]
- 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. Legionella BCYE Agar Base and the supplements 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

- The medium base and the supplements are qualitative *in vitro* diagnostics, for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association, according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- The supplements are sterilised by membrane filtration.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Before the use, consult the Safety Data Sheets.
- All laboratory specimens should be considered infectious.
- When handling *Legionella* spp., it is important to avoid aerosol formation. Thoroughly clean and disinfect all working areas
- The laboratory area must be controlled to avoid contaminants such as powder medium and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements 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

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps.

Supplements

Upon receipt, store the product in the original package at +2°C /+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/bottles) and the applied storage conditions (temperature and packaging).

16 - REFERENCES

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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.

423212 LEGIONELLA BCYE α-GROWTH SUPPLEMENT W/O CYSTEINE
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.
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.

423215 LEGIONELLA SELECTIVE SUPPLEMENT GVPC
SDS rev 3
Regulation (EU) 2020/878

Mixture containing cycloheximide

Classification

Germ cell mutagenicity, category 2	H341	Suspected of causing genetic defects.
Reproductive toxicity, category 1A	H360	May damage fertility or the unborn child.
Acute toxicity, category 3	H301	Toxic if swallowed.
Hazardous to the aquatic environment, chronictoxicity, category 3	H412	Harmful to aquatic life with long lasting effects.

Labelling
Pictogram

Signal word Danger

Hazard statement(s)

H341	Suspected of causing genetic defects.
H360	H301 Toxic if swallowed.
H412	Harmful to aquatic life with long lasting effects. Restricted to professional users.

Precautionary statements:

P201	Obtain special instructions before use.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P308+P313	IF exposed or concerned: Get medical advice / attention.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER / doctor / . . .
P264	Wash . . . thoroughly after handling.
P273	Avoid release to the environment. May damage fertility or the unborn child.

423225 LEGIONELLA AB SELECTIVE SUPPLEMENT
SDS rev 1
Regulation (EU) 2020/878

Mixture containing sodium cefazolin

Classification

Respiratory sensitization, category 1	H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Skin sensitization, category 1	H317	May cause an allergic skin reaction.

Labelling
Pictogram

Signal words: Danger
Hazard statement(s)

H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H317	May cause an allergic skin reaction.

Precautionary statement(s)

P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P280	Wear protective gloves.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER / doctor / . . .
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

TABLE OF APPLICABLE SYMBOLS

REF or **REF** Catalogue number	**LOT** Batch code	**IVD** *In vitro* Diagnostic Medical Device	Manufacturer	This side up	Store in a dry place
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Fragile	Keep away from direct light

REVISION HISTORY

Version	Description of changes	Date
Revision 8	Updated layout and content	2020/10
Revision 9	Update of “precautions and warnings”, “storage conditions and shelf life”, hazards statements	2022/04
Revision 10	Removal of obsolete classification	2023/04

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

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