

## Specification

Supplement for the isolation of *Legionella* species.

## Presentation

10 Freeze dried vial  
Vial  
with: 6 ± 0.1 g

### Packaging Details

22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.

### Shelf Life

36 months

### Storage

2-8 °C

## Composition

Composition (g/vial)

Glycine (ammonia free).....	1.5000
Vancomycin.....	0.0005
Polymyxin B sulphate.....	0.0032
Natamycin.....	0.1000
Bromocresol purple.....	0.0050
Bromthymol blue.....	0.0050

NOTE: Each vial is sufficient to supplement 500 ml Legionella BCYE Agar Base (1311).

Reconstitute the original freeze-dried vial

by adding :

Sterile Distilled Water..... 10 ml

## Description /Technique

### Description:

The Legionella BCYE Agar Base and its supplements have been shown to be optimal for Legionella culture with shorter incubation periods from environmental and clinical samples. The addition of antimicrobial agents gives selectivity to the medium. Bromothymol blue and bromocresol purple color the colonies allowing the identification of the microorganisms. ISO 11731 recommends the following procedure for the isolation of Legionella and its enumeration in water samples. The samples are concentrated by membrane filtration, diluted or inoculated directly on the plate depending on the origin and characteristics of the sample. Independent fractions of the diluted sample should be subjected to heat or acid treatments in case of a high concentration of Legionella and other bacteria. These samples are transferred to the plates with the selective culture medium chosen for Legionella.

### Technique:

Aseptically reconstitute 1 vial with 10 ml of distilled or de-ionized water. Mix gently until complete dissolution and aseptically add to 500 ml medium Legionella BCYE Agar ISO (Cat. 1311 + Cat. 6022) cooled to 50 °C. Mix well and distribute into sterile containers.

For the cultivation of legionella according to ISO 11731:

If the sample contains a high concentration of Legionella and a low concentration of interfering microorganisms:

- Directly inoculate 0,1-0,5 ml of the sample by distributing it uniformly on a plate of Agar BCYE (Cat. 1311 + Cat. 6022) and on a BCYE+AB plate.

If the sample contains a low concentration of Legionella and a low concentration of interfering organisms: - Filter the initial sample by membrane.

- Place the filter on the BCYE plate.

- Repeat the process for GVPC agar (Cat. 1311 + Cat. 6025) and / or MWY agar (Cat. 1311 + Cat. 6067).

If the sample contains a high concentration of interfering microorganisms:

- It will be inoculated directly, concentrated or diluted.

- Divide each type of sample into three portions. One of them will be used untreated, the second one will be subjected to a thermal treatment and the third will be subjected to an acid treatment.

- Inoculate 0,1-0,5 ml on GVPC agar plates and MWY agar.

If the sample contains an extremely high amount of interfering microorganisms: - It will be inoculated directly and diluted.

- Each sample is subjected to a combined thermal and acid treatment.

- Inoculate 0,1-0,5 ml on GVPC agar plates and MWY agar.

- Let the sown plates rest until the inoculum has been absorbed. Incubate at 36±2 °C for 7-10 days.

- Confirm presumptive colonies of Legionella on BCYE agar and BCYE-cys agar.

## Quality control

### Physical/Chemical control

Color : Light beige

pH: at 25°C

### Microbiological control

Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Inoculate: Practical range  $100 \pm 20$  CFU. min. 50 CFU (productivity)/  $10^4$ - $10^6$  (selectivity).Aerobiosis. Incubation at  $36 \pm 2$  °C. Reading 2 - 5 days.

Microbiological control accor. to ISO 11133:2014/A1:2018 standard

### Microorganism

*Legionella pneumophila* ATCC® 33152, WDCM 00107 (by MF)*Legionella pneumophila* ATCC® 33152*Escherichia coli* ATCC® 25922, WDCM 00013

### Sterility Control

100 ml TSB and 100 ml Thioglycollate.

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

### Growth

Good ( $\geq 70\%$ ) grey-blue coloniesGood ( $\geq 70\%$ ) grey-blue colonies

Inhibited

## Bibliography

Feeley J.C., Groman G.W., Weaver R.E., Mackel D.C..

International standard ISO 11731 water quality- Detection and enumeration of Legionella.