Reference: DSHB3078 **A.B.E. - Technical Data Sheet**

Product: # Legionella BYCE+AB Selective Supplement

Specification

Sterile selective supplement for the isolation of *Legionella* spp. from environmental water samples.

Presentation

	Packaging Details	Shelf Life	Storage
10 Freeze dried vials Vial with: 3 ± 0.1 g	22±0.25 x 55±0.5 mm glass vials, tag labelled, White plastic cap - 10 vials per box.	49 months	2-25 ºC

Composition

Compositon (vial)

Polymyxin B sulfate.....40000,00 IU
Sodium cefazolin.....4,50 mg
Pimaricin (syn Natamycin)..... 35,00 mg

Reconstitute the original freeze-dried vial
by adding :
Sterile Distilled Water.....9 ml

Description /Technique

Description:
The discovery of the causative organism of Legionnaires' disease has permitted big progress in the studies around it. New media for the culture and the enumeration *Legionella spp* have been developped in the last years.
Legionella BCYE + AB selective supplement, when added to the agar Base, gives the antibiotic support in order to obtain a selective final medium.
The selectivity is raised by the addition on sodium cefazolin that acts against Gram positive bacteria, polymyxin B that inhibits Gram negative bacteria and natamycin that are antifungal agents and inhibits the yeast growth.

Technique:
Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.
Reconstitute the 1 vial of Selective Supplement of *Legionella* BCYE + AB with 9 ml to the steril distilled water, in aseptic conditions, and add it to 500 ml of melted Legionella BCYE Agar Base cooled to 47- 50ºC supplemented before with Legionella BCYE growth Supplement. Do not overheat once supplemented.
Pour the complete medium into Petri dishes and, once solidified on a flat surface. Spread the plates by streaking methodology or by MF method.
Allow the inoculated plates to stand until the inocula has been absorbed. Invert the plates and incubate at 36 ± 2ºC for up to 2, 3, 5 -10 days. To ensure the atmosphere in the incubator is humid, place a tray of water in the bottom of the incubator. Top up this tray with fresh water (if necessary) each time the plates are examined. Incubation in an atmosphere of air with 2,5% (volume fraction) CO_2 may be beneficial for the growth of some *Legionella*, but it is not essential.
Examine the plates with a plate microscope on at least three occasions at intervals of 2,3 to 5 days during the 10-day incubation period, as *Legionella* grow slowly an can be masked by the growth of other organisms. Record the number of each type of colony present. Colonies of *Legionella* are often white-grey-blue-purple in colour, but may be brown, pink, lime-green or deep-red. They are smooth with a smooth edges and exhibit a characteristic ground-glass appearance. Under ultraviolet light colonies of several species autofluoresce brilliant white, but others are red and *L. pneumophila* appear dull green often tinged with yellow. All presumptive colonies must be confirmed by cultural, biochemical, serological or genetic methods.

Quality control

Physical/Chemical control

Color : White

Microbiological control

Reconstitute 1 vial as indicated in COMPOSITION; shake and dissolve completely
Add 1 vial to 500 ml of medium base. DO NOT HEAT once supplemented.

Aerobiosis. Incubation at 36 ± 2 ºC. Reading 3 - 5 days, up to 10 days.

Microorganism	Growth
Escherichia coli ATCC® 8739, WDCM 00012	Inhibited
Enterococcus faecalis ATCC® 19433, WDCM 00009	Inhibited
L. anisa ATCC® 35292, WDCM 00106 (by MF)	Good (≥ 50%) grey-blue colonies
L. pneumophila ATCC® 33152, WDCM 00107 (by MF)	Good (≥ 50%) grey-blue colonies

the reference medium is GVPC validated.

Sterility control

Add 5 ml of the sample to:
100 ml TSB and 100 ml Thioglycollate.
Incubation 48 hours at 30-35 ºC and 48 hours at 20-25 ºC: NO GROWTH.

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