Reference: DSHB3069 **A.B.E. - Technical Data Sheet**

Product: ***Legionella*** **BCYE Growth Supplement**

Specification

Buffering supplement with the growth factors to complete the medium Base CYE into *Legionella* BCYE Agar.

Presentation

	Packaging Details	Shelf Life	Storage
5 (Lyoph.) + 5 (Solv.) Vial with: 7,5 ± 0.3 ml	1 box with 10 vials with white plastic cap and tag labelled (5 Freeze-dried vials + 5 vials with Steril Solvent).	36 months	2-25 ºC

Composition

Compositon (g/vial)

ACES Buffer..... 5.000 g
(N-2-acetamido-2-aminoethanesulfonic acid)
Potassium hydroxide..... 1.400 g
Ferric pyrophosphate.....0.125 g
Potassium Alfa-ketoglutarate.....0.500 g
L-Cysteine HCl.....0.200 g

Reconstitute the original freeze-dried vial by adding 1 vial with:
Sterile Solvent.....7,5 ml

NOTE : Each vial is sufficient to supplement 500 ml Legionella CYE Agar Base. "In some case, crystallization can occurs in the vial. This don't affect nor quality nor solubility of the product after adding it to the medium".

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 GVPC 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 vancomicin that acts against Gram positive bacteria, polymyxin B that inhibits Gram negative bacteria and cicloheximide or 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 Growth Supplement with 7,5 ml steril solvent, in aseptic conditions, and add it to 500 ml of melted Legionella BCYE Agar Base cooled to 47- 50ºC. 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 : Yellowish / grayish

Microbiological control

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

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 36 ± 2 ºC. Reading 2 - 5 days.

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

Microorganism	Growth
Legionella pneumophila ATCC® 33152, WDCM 00107	Good (≥70%)
Legionella anisa ATCC® 35292, WDCM 00106	Good (≥70%)

Sterility control

Add 5mL of the sample to 100 mL of TSB and to 100 mL Thioglycollate.
Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

· ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press. BocaRaton. Fla. USA.
· CLESCERI, L.S., A.E. GREENBERG & A.D. EATON (1998) Standard methods for the examination of water and wastewater. 9-106. 20th edition. APHA-AWWA-WEF. Washington DF, USA.
· EDELSTEIN, P.H., (1981) Improved semiselective medium for the isolation of *Legionella pneumoniae* from contaminated clinical and environmental specimens. J. Clin Microbiol. 14(3):298.
· FEELEY, J.C., R.J. GIBSON, G.W. GORMAN, N.C. LANGFORD, J.K. RASHEED, C.D. MACKEL, & W.B. BAINE (1979) Charcoal-Yeast Extract Agar: Primary isolation medium for *Legionella pneumophila*. J. Clin. Microbiol. 10(4) 437.
· ISO 11731 Standard (2017) Water Quality - Enumeration of *Legionella*.
. ISO 11133:2014/ Adm 1:2018/ Adm1 :2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· MacFADDIN, J.F. (1985) Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria.
· PASCULLE, A.W., J.C. FEELEY, R.J. GIBSON, L.G. CORDES, R.L. MYEROWITZ, C.M. PATTON, G.W. GORMAN, C.L. CARMACK, J.W. EZZELL & J.N. DOWLING (1980) Pittsburgh pneumonia agent: Direct isolation from human lung tissue. J. Infect. Dis., 141:727.
. UNE-EN ISO 11133 (2014). Microbiología de los alimentos para consumo humano, alimentación animal y agua.-Preparación, producción, conservación y ensayos de rendimiento de los medios de cultivo.
· WARD, K.W. (1995) Processing and interpretation of specimens for *Legionella spp.* In "Clinical Microbiology Procedures Handbook" Chap. 12.1 edited b H.D. Isenberg. ASM Press. Washington DC, USA.