

TECHNICAL DATA SHEET

Article No. 8861

Legionella BCYE Growth Supplement

SPECIFICATION

Growth supplement to complete the BCYE medium base.

COMPOSITION (G/VIAL)

ACES Buffer	5.000 g
(N-2-acetamido-2-aminoethanesulfonic acid)	
Potassium hydroxide	1.400 g
Iron(III) pyrophosphate	0.125 g
Potassium α -ketoglutarate	0.500 g
L-Cysteine hydrochloride	0.200 g

Reconstitute the original freeze-dried vial by adding:

Sterile solvent..... 7,5 ml

Each vial is sufficient to supplement 500 ml Legionella BCYE Agar (base) (Art. no. 8811).
5 vials with freeze-dried supplement plus 5 vials sterile solvent per box.

In some cases, crystallization can occur in the vial. This does not affect the quality nor solubility of the product after adding it to the medium.

DESCRIPTION/TECHNIQUE

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 developed in the last years. Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Reconstitute the vial with 9 ml of the included diluent, in aseptic conditions, and add it to 500 ml of melted Legionella BCYE Agar (base) cooled to 50 °C. 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 spiral method.

Incubate the plates, when the inoculum has been completely absorbed, in aerobic atmosphere at 36 °C for 4-10 d. To ensure the atmosphere in the incubator is humid, place a tray of water in the bottom of the incubator. Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample or the specifications.

Examine the plates with a plate microscope on at least three occasions at intervals of 2-4 d during the 10-day incubation period, as *Legionella* grow slowly and can be masked by the growth of other organisms.

Record the number of each type of colony present. After incubation, count all the colonies that have appeared on the surface of the agar.



Colonies of *Legionella* are often white-grey-blue-purple in colour, but may be brown, pink, lime-green or deep-red. Presumptive isolation must be confirmed by further microbiological and biochemical tests.

QUALITY CONTROL

- Physical/chemical control: Colour yellowish/grayish
pH at 25 °C
- Microbiological control: Reconstitute 1 vial as indicated, shake and dissolve completely.
Add 1 vial to 500 ml of medium base. Do not heat once supplemented.
Aerobiosis. Incubation at 36 ±2 °C. Reading 3-5 d, up to 10 d.

Microorganism	Growth	Remarks
<i>Legionella anisa</i> ATCC® 35292 (by MF)	Good (≥50 %) grey-blue colonie	None
<i>Legionella pneumophila</i> ATCC® 33152 (by MF)	Good (≥50 %) grey-blue colonie	None

- Sterility control: Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: No growth.
Check at 7 d after incubation at the same conditions.

REFERENCES

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STORAGE

2-25 °C

SHELF LIFE

36 months from date of production.

