



Reference : 01-687

Scharlau Microbiology - Technical data sheet

Product :
Legionella BCYE AGAR BASE

Also known as

CYE

Specification

Solid medium base used for the detection, isolation and enumeration of *Legionella* from water, according to ISO standard 11731:2017.

Formula * in g/L

Activated charcoal 2.00

Yeast extract 10.00

Agar 15.00

Final pH 6.8 ±0.2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 13,5 g of powder in 500 mL of distilled water and bring to the boil dissolving completely. Sterilize by autoclaving at 121 °C for 15 minutes. Allow to cool to 50 °C and add aseptically a reconstituted vial (Ref. No. 06-137LYO1) of *Legionella* BCYE Growth Supplement. Mix gently and pour into Petri dishes. The final pH at 25 °C should be adjusted to 6.8 ± 0.2. If higher selectivity is desired, one vial of the GVPC Selective Supplement for *Legionella* (Ref. 06-138LYO1) or MWY Selective Supplement for *Legionella* should be used.

If the control medium BCYE - Cys is desired it can be obtained by the addition of a reconstituted vial of (Art. No. 06-134LYO1) *Legionella* BCYE w/o Cysteine Growth Supplement to the sterile, melted and cooled *Legionella* BCYE Agar Base.

**Product :**
Legionella BCYE AGAR BASE**Description**

The actual formulation of this medium is according to the ISO Standards 11731: 2017, but BCYE Agar is based in a modification of a previously described media. In 1979 Feeley and collaborators described Charcoal Yeast Extract (CYE) Agar as a modification of the F-G Agar. They replaced the starch in the F-G Agar with activated charcoal and substituted yeast extract for casein hydrolysate, resulting in a better recovery of *Legionella pneumophila*. Pasculle, in 1980, reported that CYE Agar could be improved by buffering the medium with ACES buffer and a year later Edelstein increased the sensitivity of the medium by adding a-ketoglutarate which is the present formulation (BCYE Agar).

The medium consist of a Medium base supplemented with growth factors (BCYE Agar) and the Selective Medium supplemented with inhibitors of undesirable accompanying flora. The yeast Extract supplies the basic nutrients as the medium contains no fermentable carbohydrates. L-Cysteine, Ferric pyrophosphate and a-ketoglutarate are incorporate to satisfy the specific nutritional requirements of *Legionella* species.

The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect CO₂ and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth. The selectivity is raised by the addition on Vancomycin / Sodium cefazolin they acts against gram-positive bacteria, Polymyxin B that inhibits gram-negative bacteria, anisomycin that has a broad spectrum of activity and Cycloheximide or Natamycin that as antifungal agents inhibits the yeast growth.

Supplements to complete to the medium Base:

-*Legionella* BCYE Growth Supplement (Art. No. 06-137LYO1)

Vial Contents:

Necessary amount for 500 mL of complete medium.

| | |
|--------------------------------|---------|
| ACES Buffer..... | 5,000 g |
| Potassium hydroxide..... | 1,400 g |
| Ferric pyrophosphate..... | 0,125 g |
| L-Cysteine HCl..... | 0,200 g |
| Potassium a-ketoglutarate..... | 0,500 g |
| Sterile Solvent | |

-*Legionella* GVPC Selective Supplement (Art. No. 06-138LYO1)

Vial Contents:

Necessary amount for 500 mL of complete medium.

| | |
|-----------------------------|-------------|
| Vancomycin..... | 0,50 mg |
| Polymyxin B sulfate..... | 40000,00 IU |
| Cycloheximide..... | 40,00 mg |
| Glycine (ammonia free)..... | 1,50 g |
| Distilled water (Solvent) | |

-*Legionella* BCYE w/o Cysteine NO Growth Supplement (Ref.06-134LYO1).

Vial Contents:

Necessary amount for 500 mL of complete medium.

| | |
|--------------------------------|---------|
| ACES Buffer..... | 5,000 g |
| Potassium hydroxide..... | 1,400 g |
| Ferric pyrophosphate..... | 0,125 g |
| Potassium a-ketoglutarate..... | 0,500 g |
| Sterile Solvent. | |

Other alternatives (check availability)

-*Legionella* MWY Selective Supplement:

Necessary amount for 500 mL of complete medium.

| | |
|-----------------------------|-------------|
| Glycine (ammonia free)..... | 1,50 g |
| Polymyxin B sulfate..... | 25000,00 IU |
| Vancomycin Hcl..... | 0,50 mg |
| Anisomycin..... | 40,00 mg |



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Product :
Legionella BCYE AGAR BASE

Bromothymol blue..... 5,00 mg
Bromocresol purple..... 5,00 mg
Distilled water (Solvent)

-*Legionella* BCYE + AB Selective Supplement

Vial Contents:

Necessary amount for 500 mL of complete medium.

Polymyxin B sulfate..... 40000,00 IU

Technique

Refers to ISO 11731:2017 or other standard procedures to obtain isolated colonies from specimens and samples. Allow the inoculated plates to stand until the inocula has been absorbed. Invert the plates and incubate à 36 ±2 °C for up to 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) CO₂ may be beneficial for the growth of some Legionella, but it is not essential.

Examine the plates with a plate microscope on à least three occasions à intervals of 2 to 4-5 days during the 10 days incubation period, as Legionella grows slowly and 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 smooth edges and exhibit a characteristic ground-glass appearance. Under ultraviolet light colonies of several species are autofluorescent 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

Incubation temperature: 36 °C ± 2

Incubation time: 2 - 5 - 10 days

Inoculum: Practical range 100 ±20 CFU. min. 50 CFU (productivity)/10⁴ -10⁵ CFU (selectivity), according to ISO 11133:2014/Amd 1:2018.

Microorganism

Legionella pneumophila ATCC® 33152

Escherichia coli ATCC® 8739

Pseudomonas aeruginosa ATCC® 9027

Enterococcus faecalis ATCC® 19433

Legionella anisa ATCC® 35292

Growth

Productivity > 0.50

Partial Inhibition

Partial Inhibition

Inhibited

Productivity > 0.50

Remarks

Grey - white colonies (2-5 d)

w. supplement GVPC (3 d)

w. supplement GVPC (3 d)

w. supplement GVPC (3 d)

Grey - white colonies (5-10 d)

References

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- 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.
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- ISO 11133:2014/ Adm 1:2018. 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.
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- 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.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).