TECHNICAL DATA SHEET

Article no. : 8811.0500

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 heat to boiling until completely dissolved. Sterilize by autoclaving at 121 °C for 15 minutes. Allow to cool down to 47-50 °C and add aseptically a reconstituted vial of *Legionella* BCYE Medium Supplement (Art. no. 8861). Mix gently and pour into Petri dishes. The final pH at 25 °C should be 6.8 ±0.2.
If a selective medium for *Legionella* is desired, it can be obtained by addition of one vial of *Legionella* GVPC Selective Supplement (Art. no. 8820) to 500 ml of BCYE Medium (47-50 °C).

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DESCRIPTION

The actual formulation of this medium is according to ISO 11731, but BCYE Agar is based on a modification of a previously described media. In 1979 Feeley et al. 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 α-ketoglutarat which is the present formulation (BCYE Agar).
The medium consists 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, iron(III) pyrophosphate and α-ketoglutarat are incorporated to satisfy the specific nutritional requirements of *Legionella species*.
The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect CO_2 and modify surface tension. The addition of the buffer helps maintain the proper pH for optimal growth. The selectivity is increased by the addition of vancomycin and polymyxin B which inhibit Gram positive bacteria and cycloheximide or natamycin which are antifungal agents and inhibits the yeast growth.

Necessary supplements:

Legionella BCYE Medium Supplement (Art. no. 8861)
Necessary amount for 500 ml of complete medium.
ACES Buffer 5.000 g
Potassium hydroxide 1.400 g
Ferric pyrophosphate 0.125 g
Potassium α-ketoglutarat 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

Legionella GVPC Selective Supplement (Art. no. 8820)
Necessary amount for 500 ml of complete medium.
Glycine (ammonia free) 1.5000 g
Vancomicin 0.0005 g
Polymyxin B sulphate 40000 IU
Cyclohexymide 0.0400 g

Reconstitute the original freeze-dried vial
by adding :
Sterile Distilled Water 10 ml

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 at 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) 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 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.

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QUALITY CONTROL

Incubation Temperature: 36 ºC ± 2
Incubation Time: 2 - 5 - 10 days
Inoculum: Practical range 100 ±20 CFU. min. 50 CFU (productivity)/10^4-10^6 CFU (selectivity), according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Legionella pneumophila ATCC® 33152	Productivity > 0.50	Grey - white colonies (2-5 d)
Escherichia coli ATCC® 8739	Partial Inhibition	w. supplement GVPC (3 d)
Pseudomonas aeruginosa ATCC® 9027	Partial Inhibition	w. supplement GVPC (3 d)
Enterococcus faecalis ATCC® 19433	Inhibited	w. supplement GVPC (3 d)
Legionella anisa ATCC® 35292	Productivity > 0.50	Grey - white colonies (5-10 d)

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STORAGE

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

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