Legionella BCYE Agar Base

Art. No. 01-687

Also known as

CYE

Specification

Solid medium base used for the detection, isolation and enumeration of *Legionella* from water according to the ISO standards 11731:1998 and 11731-2:2004.

Formula* in g/L

Activated Charcoal	2,00
Yeast Extract	10,00
Agar	15,00

Final pH 6,9 ± 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 47-50°C and add aseptically a reconstituted vial (Art. No. 06-137-LYO) of *Legionella* BCYE Growth Supplement. Mix gently and pour into Petri dishes. If a selective medium for *Legionella* is desired, it can be obtained by the addition of a vial (Art. No. 06-138-LYO) of *Legionella* GVPC Selective Supplement to 500 mL of BCYE Medium melted and cooled to 47-50°C.

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

Description

The actual formulation of this medium is according to the ISO Standards 11731 and 11731-2, 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 α-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 α-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 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.

Technique

Refer to the ISO Standards 11731 and 11731-2 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 ± 1°C for up to 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 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.

Necessary supplements

Legionella BCYE Growth Suplement (Art. No. 06-137-LYO)

ACES Buffer	3,600 g
Potassium hydroxide	1,400 g
Ferric pyrophosphate	0,125 g
L-Cysteine HCl	0,200 g
Potassium α–ketoglutarate	0,500 g

Distilled water (Solvent)

Legionella GVPC Selective Suplement (Art. No. 06-138-LYO) and

Vancomycin	0,50 mg
Polymyxin B sulfate	40000,00 IU
Cycloheximide	40,00 mg
Glycine (ammonia free)	1,50 g

Distilled water (Solvent)

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***Legionella* BCYE w/o Cysteine NO Growth Suplement (Art. No. 06-134-LYO)**

Vial Contents:

Necessary amount for 500 mL of complete medium.

ACES Buffer	3,600 g
Potassium hydroxide	1,400 g
Ferric pyrophosphate	0,125 g
Potassium α–ketoglutarate	0,500 g

Distilled water (Solvent)

References

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0

Incubation time: 3 - 10 days

Inoculum: 150-300 CFU (Productivity) // 1.000-10.000 CFU (Selectivitu). Spiral Plate Method (ISO 11133-1/2)

Microorganism	Growth	Remarks
Legionella pneumophila ATCC 33152	Good	Grey - white colonies
Staphilococcus epidermidis ATCC 12228	Inhibited	-