## 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 121ºC for 15 minutes. Allow to cool to 47-50ºC and add aseptically a reconstituted vial (Ref. No. DSHB3069) 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 a selective medium is desired it may be obtained by additionally adding a vial of GVPC Selective Supplement for Legionella (Ref.DSHB3070).

## 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 $CO_2$ 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.

**Necessary supplements to complete to the medium Base:**

-*Legionella* BCYE Growth Supplement (Art. No. DSHB3069)
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.DSHB3070)
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)

## 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) $CO_2$ 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 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. MF methods & Spiral Plate methods.

| Microorganism | Growth | Remarks |
|---|---|---|
| *Legionella pneumophila* ATCC® 33152 | Productivity > 0.50 | Grey - white colonies (2-5 d) |
| *Leg. pneumophila* ATCC® 33152 (MF method) | Productivity > 0.50 | Grey - white colonies (2-5 d) |
| *Legionella anisa* ATCC® 35292 | Productivity > 0.50 | Grey - white colonies (5-10 d) |
| *Legionella anisa* ATCC® 35292 (MF method) | Productivity > 0.50 | Grey - white colonies (5-10 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) |

## References

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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).