

## LEGIONELLA CYE AGAR BASE ISO 11731-2

### CAT N°: 21311

Selective medium for the cultivation of *Legionella*

### FORMULA IN g/l

Yeast Extract	10.00	Bacteriological Agar	13.00
Activated Charcoal	2.00		

**Final pH 6.9 ± 0.2 at 25°C**

### PREPARATION

Suspend 2.5 grams of the medium in 90 ml of distilled water. Heat until boiling and until the medium is completely dissolved. Distribute into appropriate containers and sterilize in autoclave at 121 °C for 15 minutes. Cool to 45 - 50°C and aseptically add 1 vial of Legionella CYE Growth Supplement (Cat. 26022), previously reconstituted in 10 ml of sterile distilled water and, if desired, also add 1 ml of 1 vial of LEGIONELLA GVPC SUPPLEMENT (Cat. 26025), previously reconstituted with 5 ml of sterile 1:1 water/acetone (see Preparation of Cat. 26025). Mix well and distribute into appropriate containers. The prepared medium should be stored at 8-15°C. The color is black.

The dehydrated medium should be homogeneous, free-flowing and black in color. If there are any physical changes, discard the medium.

#### Legionella BCYE Growth Supplement (Cat. 26022)

(For 100 ml of base medium)

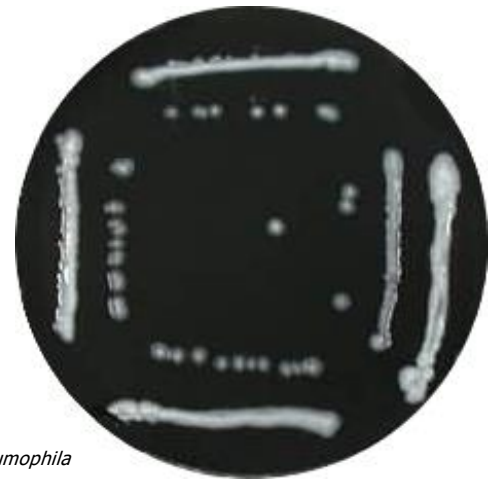
Potassium Hydroxide.....	200 mg
L-Cysteine HCl .....	40 mg
Ferric Pyrophosphate.....	25mg
Buffer ACES.....	1.0 g
Alpha Ketoglutarate.....	0.1g

#### Legionella GVPC Growth Supplement (Cat. 26025)

(For 500 ml of base medium)

Polymyxin B Sulfate .....	40000 IU
Glycine .....	1.5g
Cycloheximide .....	40mg
Vancomycin .....	0.5mg

**Caution:** Legionella GVPC Supplement contains cycloheximide and is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.



*Legionella pneumophila*  
ATCC 33153

### USES

LEGIONELLA CYE AGAR BASE and its supplements have proven to be optimum for the cultivation of *Legionella* with shorter incubation periods from environmental and clinical samples.

Yeast extract provides vitamins, particularly of the B-group, and other growth co-factors. L-Cysteine provides the required nutritional source. Activated charcoal is a protective agent neutralizing and absorbing toxic metabolites produced by bacterial growth. It decomposes hydrogen peroxide, a toxic metabolic product, and can also collect CO<sub>2</sub> and modify surface tension.

Inoculate sample and incubate at 35 ± 2°C for 24 - 72 hours and up to 10 days. Colonies suspected of being *Legionella* are subcultured to Tryptone Soy Agar (Cat. 1138) with 5% sheep blood.

Isolates that grow on CYE Agar but fail to grow on Tryptone Soy Agar with blood may be presumed to be *Legionella*. Confirmation to be made by biochemical and serological tests. The following criteria are suggested:

- 1.Colonies which have characteristic morphology
- 2.Isolates should not grow on Tryptone Soy Agar with blood
- 3.Organisms show gram morphology

Colony Morphology after incubation at 35°C after 48 – 60 hours:

*L. pneumophila*: 1 - 2 mm diameter (increases after further incubation). White, glistening, circular, smooth, raised with entire edge.

*L. gormanii*: 1 - 2 mm diameter. Cream, mucoid, slightly raised.

Other Legionellae: 1 - 2 mm diameter (increases after further incubation). White, glistening, circular, smooth, raised with entire edge.

The International standard ISO 11731 recommends the following steps for the confirmation of presumptive *Legionella* colonies: Subculture to CYE without Cysteine at least five colonies characteristic of *Legionella* at random for each sample for subculture onto plates of CYE and CYE without Cysteine medium. If different types of presumptive *Legionella* colonies are present, make sure to select at least two colonies from each type. Subculture each colony onto plates of both media. Incubate at  $36 \pm 2^\circ\text{C}$  for at least 2 days. Regard those colonies which grow on CYE but fail to grow on CYE without Cysteine medium as *Legionella*. Record the results for each plate.

Nutrient agar or blood agar medium may be used instead of CYE without Cysteine medium.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with both supplements added, from type cultures after incubation at a temperature of  $35 \pm 2^\circ\text{C}$  and observed after 24 - 72 hours and up to 10 days. .

Microorganisms	Growth	Colony Color
<i>Legionella pneumophila</i> ATCC 33153	Good	White
<i>Escherichia coli</i> ATCC 25922	Inhibited	-
<i>Staphylococcus epidermidis</i> ATCC 12228	Inhibited	-

## STORAGE

Once opened keep powdered medium closed to avoid hydration.



## BIBLIOGRAPHY



Feeley J.C., Groman G.W., Weaver R.E., Mackel D.C..  
International standard ISO 11731 water quality- Detection and enumeration of *Legionella*