

**PRODUCT CODE: 455378**

## **Legionella Selective Agar (ISO 11731) (Prepared Plate (@ 90 mm)) for microbiology**

### **Specification**

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

### **Presentation**

<b>20 Prepared Plates</b>	<b>Packaging Details</b>	<b>Shelf life</b>	<b>Storage</b>
90 mm with: 21 ± 2 ml.	1 box with 2 packs of 10 plates/pack. Single cellophane.	3 months	2-14°C
<b>120 Prepared plates</b>	<b>Packaging Details</b>	<b>Shelf life</b>	<b>Storage</b>
90 mm with: 22 ± 1 ml.	1 box with 2 packs of 10 plates/pack. Single cellophane.	3 months	2-14°C

### **Description and Technique**

#### *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<sub>2</sub> 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 or 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.

*Technique*

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For 20 Prepared plates:

Refer to the ISO Standards 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  $36 \pm 2^\circ\text{C}$  for up to 2, 3, 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<sub>2</sub> 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 2,3 to 5 days during the 10-day incubation period, as *Legionella* grow 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 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.

Note: If the medium is used with the membrane filter method, the colour and growth of the colonies may be effected. It is advisable to perform validation of the membrane filter used, by the technical.

For 120 Prepared plates:

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  $36 \pm 1^\circ\text{C}$  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<sub>2</sub> 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 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 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.

Note: If the medium is used with the membrane filter method, the colour and growth of the colonies may be effected. It is advisable to perform validation of the membrane filter used, by the technical.

## Quality control

### For both presentations:

Physical/Chemical control	Microbiological control	Sterility control
Color: Black. pH: 6.8 ± 0.2 at 25°C	Spiral Spreading /MF Methods; Practical range 100±20 CFU; Min. 50 CFU (Productivity) / 10 <sup>4</sup> -10 <sup>6</sup> CFU (Selectivity). Microbiological control according to ISO 11133:2014 Aerobiosis. Incubation at 36 ± 2 °C. Reading 3 - 5 days, up to 10 days.	Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH  Check at 7 days after incubation in same conditions

Microorganism	Growth
<i>Ps. aeruginosa</i> ATCC® 9027, WDCM 00026	Partial Inhibition.
<i>Escherichia coli</i> ATCC® 8739, WDCM 00012	Inhibited.
<i>Enterococcus faecalis</i> ATCC® 19433, WDCM 00009	Inhibited.
<i>L. anisa</i> ATCC® 35292, WDCM 00106 (by MF)	Good (≥ 50%) grey-blue colonies.
<i>Legionella anisa</i> ATCC® 35292, WDCM 00106	Good (≥ 50%) grey-blue colonies.
<i>L. pneumophila</i> ATCC® 33152, WDCM 00107 (by MF)	Good (≥ 50%) grey-blue colonies.
<i>Legionella pneumophila</i> ATCC® 33152, WDCM 00107	Good (≥ 50%) grey-blue colonies
Membrane filter NALGDS0525-6045, batch used:	F6NA27518

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