

**Legionella (B.C.Y.E.) Agar (with ACES and  $\alpha$ -Ketoglutaric Acid)**

Medium used for the isolation and cultivation of Legionella spp.

- **CONTENTS (Liter)**

Charcoal	1.5 g
Yeast Extract	10.0 g
Aces	6.0 g
A-Ketoglutaric Acid	1.0 g
Potassium Hydroxide	1.5 g
Agar	17.0 g
Final pH = 7.0 $\pm$ 0.2 at 25°C	

- **PROCEDURE**

Suspend 37.0 G of powder in 900 mL of distilled or deionized water. Sterilize by autoclave at 121°C for 15 minutes. Cool to 45 - 50°C in water bath. Aseptically add 10 vials of Legionella Growth supplement (MB-L1858). If necessary, aseptically add 10 vials of Legionella (B.M.P.A.) supplement (MB-L1802) or 2 vials of Legionella (G.V.P.C.) supplement (MB-L1808) or 10 vials of Legionella (M.W.Y.) supplement (MB-L1819). Mix well. Pour into petri dishes.

**Legionella Growth supplement**

1 vial contents (each vial is sufficient for 100 mL of medium)

L-Cysteine	0.04 g
Ferric Pyrophosphate	0.025 g

**Legionella (B.M.P.A.) supplement**

1 vial contents (each vial is sufficient for 100 mL of medium)

Anisomycin	0.008 g
Cefamandole	0.0004 g
Polymyxin B	8,000 IU

**Legionella (G.V.P.C.) supplement**

1 vial contents (each vial is sufficient for 500 mL of medium)

Cycloheximide	0.04 g
Glycine	1.5 g
Polymyxin B	40,000 IU
Vancomycin	0.0005 g

**Legionella (M.W.Y.) supplement**

1 vial contents (each vial is sufficient for 100 mL of medium)

Anisomycin	0.008 g
Glycine	0.3 g
Polymyxin B	5,000 IU
Vancomycin	0.0001 g
Bromthymol Blue	0.001 g
Bromcresol Purple	0.001 g

## • INTERPRETATION

Legionella (B.C.Y.E.) Agar (with ACES and  $\alpha$ -Ketoglutaric Acid) is used for the isolation and cultivation of Legionella spp. Charcoal neutralizes toxic products in the medium. Yeast extract provides the nitrogen, minerals and vitamins. ACES and potassium hydroxide get a stable pH for optimal growth.  $\alpha$ -Ketoglutarate is added to stimulate the growth of Legionella spp. Agar is the solidifying agent. L-Cysteine and ferric pyrophosphate provide nutritional requirements of Legionella species.

## • TECHNIC

Inoculate the specimen using a sterile loop to the medium. Incubate at  $36 \pm 1^\circ\text{C}$  for 3 - 5 days up to 7 days under appropriate condition, humidified atmosphere. Refer appropriate references for recommended test procedure.

## • QUALITY CONTROL FOR USE

### Dehydrated medium

Appearance: free-flowing, homogeneous

Color: dark gray-black

### Prepared medium

Appearance: opaque

Color: black

Incubation conditions:  $36 \pm 1^\circ\text{C}$  / 3 - 5 days up to 7 days under appropriate condition, humidified atmosphere

Microorganism	ATCC	Inoculum CFU	Growth	Characteristics
<i>Legionella pneumophila</i>	33152	50-300	good	grayish-blue colonies
<i>Legionella busanensis</i>	BAA-518	50-300	good	grayish-blue colonies

## • STORE

The powder is very hygroscopic. Store the powder at room temperature, in a dry environment, in its original container tightly closed and use it before the expiry date on the label. Store prepared medium at  $2 - 8^\circ\text{C}$ .

## • REFERENCES

- Dennis, P.J.L. (1988). Isolation of legionellae from environmental specimens.
- BSI Document Determination of Legionaellae in water and related materials. Method for their detection and enumeration. July 1989 DRAFT DOCUMENT. 89/53406.

## • PACKAGE

Cat. No : MB-L1049 Legionella (B.C.Y.E.) Agar (with ACES and $\alpha$ -Ketoglutaric Acid)	500 G
--	-------