

## LEGIONELLA AGAR (7307)

### Intended Use

**Legionella Agar** is used for the isolation of *Legionella* spp.

### Product Summary and Explanation

In 1977, McDade et al. identified *Legionella pneumophila* as the causative agent of Legionnaires' disease, a multisystem disease manifested primarily by pneumonia.<sup>1,2</sup> In 1978 a new medium, F-G Agar, resulted in improved growth of *L. pneumophila*, a very fastidious organism.<sup>3</sup> Freely et al. modified F-G Agar by substituting yeast extract as a vitamin source and replacing starch with activated charcoal, producing Charcoal Yeast Extract (CYE) Agar.<sup>4</sup> In 1980, Pasculle et al. reported that CYE Agar could be improved by the addition of ACES (N-2-acetamido-2-aminoethane sulfonic acid) buffer.<sup>5</sup> One year later, Edelman further increased the sensitivity of the medium by adding the potassium salt of alpha-ketoglutaric acid.<sup>6</sup>

### Principles of the Procedure

Yeast Extract provides sources of nitrogen, carbon, and vitamins in Legionella Agar. Activated Charcoal decomposes hydrogen peroxide, a metabolic product toxic to *Legionella* spp., and may also collect carbon dioxide and modify surface tension. ACES Buffer is added to maintain the proper pH for optimal growth.  $\alpha$ -Ketoglutarate stimulates organism growth. Agar is the solidifying agent. Legionella Agar is supplemented with L-Cysteine, an essential amino acid, and Ferric Pyrophosphate, an iron supplement. Both supplements are incorporated to satisfy specific nutritional requirements of *Legionella* spp. Selective agents can be added if necessary.

### Formula / Liter

Yeast Extract .....	11.5 g
Charcoal, Activated .....	1.5 g
ACES Buffer .....	6 g
$\alpha$ -Ketoglutarate .....	1 g
Agar .....	17 g

Final pH: 6.85  $\pm$  0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Supplements / 10 mL

L-Cysteine (4%), sterile  
Ferric Pyrophosphate (2.5%), sterile

### Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

### Directions

1. Suspend 37 g of the medium in one liter of purified water.
2. Adjust pH to 6.8 – 6.9 with 1N KOH.
3. DO NOT heat prior to sterilization.
4. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C.
5. Aseptically add 10 mL of a sterile solution of L-Cysteine (4%) and Ferric Pyrophosphate (2.5%).
6. Mix and determine pH. If necessary, aseptically adjust pH to 6.85 - 7.0 with a sterilized solution of 1N HCl or 1N KOH. Add inhibitor solutions if required. Dispense with agitation.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing and grey-black.

**Prepared Appearance:** Prepared medium is black and opaque.

**Expected Cultural Response:** Cultural response on Legionella Agar incubated aerobically at 35 ± 2°C under 7 – 10% CO<sub>2</sub> and examined for growth after 72 - 96 hours.

Microorganism	Approx. Inoculum (CFU)	Response
<i>Legionella bozemanii</i> ATCC® 33217	10 - 300	Growth
<i>Legionella dumoffii</i> ATCC® 33279	10 - 300	Growth
<i>Legionella pneumophila</i> ATCC® 33152	10 - 300	Growth

The organisms listed are the minimum that should be used for quality control testing.

### **Test Procedure**

Culture the organism immediately upon arrival to the laboratory. Culture specimens from swabs by rolling the swab over a third of the agar surface. Streak remainder of the plate to obtain isolated colonies. Incubate inoculated plates at 35 ± 2°C for a minimum of 3 days. Growth is usually visible within 3 - 4 days, but can take up to 2 weeks.

### **Results**

*Legionella pneumophila* produces small to large, smooth, colorless to pale, blue-grey, slightly mucoid colonies that fluoresce yellow-green under longwave UV light. A gram stain, biochemical tests, and serological procedures should be performed for confirmation of *L. pneumophila*.

### **Storage**

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

### **Expiration**

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if it is not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### **Limitations of the Procedure**

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Biochemical tests and serological procedures must be performed to confirm presence of *L. pneumophila*.

### **Packaging**

<b>Legionella Agar</b>	<b>Code No.</b>	<b>7307A</b>	<b>500 g</b>
		<b>7307B</b>	<b>2 kg</b>
		<b>7307C</b>	<b>10 kg</b>

### **References**

1. McDade, Shepard, Fraser, Tsai, Redus, Dowdle and the Laboratory Investigation Team. 1977. N. Engl. J. Med. **297**:1197.
2. Edelstein. 1985. *In* Lennette, Balows, Hausler and Shadomy (eds.). Manual of clinical microbiology, 4<sup>th</sup> ed. ASM. Washington, D.C.
3. Freely, Gorman, Weaver, Mackel and Smith. 1978. J. Clin. Microbiol. **8**:320.
4. Freely, Gibson, Gorman, Lansford, Rasheed, Mackel and Baine. 1979. J. Clin. Microbiol. **10**:437.
5. Pasculle, Freely, Gibson, Cordes, Myerowitz, Patton, Gorman, Carmack, Ezzell and Dowling. 1980. J. Infect. Dis. **141**:727.
6. Edelstein. 1981. J. Clin. Microbiol. **14**:298.

### **Technical Information**

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.