

## 585: BCYE AGAR

This recipe contains strain-specific modifications for *Legionella shakespearei* DSM 23087 \*

Final volume: 1000 ml

OXOID Legionella CYE-Agar base (with Legionella BCYE supplement) ml

1. Dehydrated media preparations are available from media distributors.
2. We use OXOID Legionella CYE-Agar base with Legionella BCYE supplement.
3. For solubilization of the lyophilized pellet use standard liquid media. Although Legionellae do not grow in those media they are suitable for resuspension and transfer onto agar plates.
4. Note: Alternatively use CYE-ACES AGAR

\* Microaerophilic, needs high humidity

## CYE-ACES AGAR

<b>Solution A</b>	490.00	ml
<b>Solution B</b>	490.00	ml
<b>Solution C</b>	10.00	ml
<b>Solution D</b>	10.00	ml

Add Cysteine-HCl, then ferric pyrophosphate solution to solution A. Adjust the complete medium to pH 6.9 ± 0.5 at 50°C with sterile 1.0 N KOH or HCl. The pH of the medium is critical. Finally, add solution B. Swirl medium in flask during dispensing to petri dishes or tubes in order to keep charcoal suspended.

### Solution A

Yeast extract (Difco 0127)	10.00	g
ACES (N-2-acetamido-2-aminoethane-sulfonic acid)	10.00	g
Activated charcoal (Sigma C 5510)	2.00	g
Distilled water	490.00	ml

Dissolve ACES and bring to pH 6.9 by slowly adding 1 N KOH. Add the other components and dissolve by boiling. Autoclave at 121°C for 15 minutes. Cool to 50 - 55°C.

### Solution B (from medium 585)

Agar	15.00	g
Distilled water	490.00	ml

## 585: BCYE AGAR

Autoclave at 121°C and cool to 50 - 55°C.

### **Solution C** (from medium 585)

L-Cysteine HCl x H <sub>2</sub> O	0.40	g
Distilled water	10.00	ml

Prepare a fresh solution and filter-sterilize it.

### **Solution D** (from medium 585)

Fe <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub>	0.25	g
Distilled water	10.00	ml

1. Prepare a fresh solution by heating to 50 - 55°C in a water-bath. Filter-sterilize solution separately.
2. Please note: Soluble ferric pyrophosphate must be kept dry and in the dark. Do not use if chemical loses its green colour and becomes brown or yellow.