# Modified Buffered Charcoal Agar Base

**M892**

Modified Buffered Charcoal Agar Base with added supplements is used for selective cultivation of *Legionella* species from clinical and other specimens.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Proteose peptone | 10.000 |
| Charcoal activated | 2.000 |
| ACES buffer | 10.000 |
| alpha-Ketoglutarate monopotassium salt | 1.000 |
| Agar | 17.000 |
| Final pH ( at 25°C) | 6.9±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 20 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add sterile rehydrated contents of one vial of Legionella Supplement (FD041A and FD040). Mix well and pour into sterile Petri plates with constant agitation to ensure that charcoal particles are evenly distributed. For additional selectivity , Legionella Selective Supplements (FD017, FD037 and FD038) may be added.

## Principle And Interpretation

*Legionella* species are non-spore forming, narrow, gram-negative rods. *Legionella* causes pneumonia (Legionnaires disease) (1) or a milk, febrile disease (Pontiac fever). They do not oxidize or ferment carbohydrates in conventional media or grow on sheep blood agar. Growth is much better and more rapid on Buffered Charcoal Yeast Extract Agar (3, 4). Amino acids are the major sources of energy for *Legionella* . The amino acid L-cystine holds an absolute requirement as it plays major role in growth metabolism of *Legionella* (2). This amino acid as well as ferric pyrophosphate helps for the growth of *Legionella* . Modified Buffered Charcoal Agar is similar to Buffered Charcoal Yeast extract Agar Base except that the yeast extract is replaced by proteose peptone. This medium is recommended for isolation and cultivation of *Legionella* species from clinical and environmental specimens. The medium was formulated by Feeley et al (5) and Edelstein (7) modified it further.

The media contains charcoal, which acts as a detoxicant. Proteose peptone acts as a rich source of vitamins, nitrogen as well as carbon. ACES Buffer maintains optimal pH for growth while L-cystine hydrochloride; ferric pyrophosphate and a-Ketoglutarate stimulate growth of *Legionella* species. For selective isolation, antibiotic supplements can be used to suppress contaminating microorganisms. Legionella Selective Supplement II (CCVC) (FD037) containing cephalothin, colistin, vancomycin and cycloheximide (8) or Legionella Selective Supplement IV (MWY) (FD040) containing glycine, polymyxin B, anisomycin, vancomycin, bromothymol blue and bromocresol purple (6) are often used. Wear gown, mask and gloves while handling *Legionella* cultures. Work in a safety hood.

## Quality Control

**Appearance**
Grey to black homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.7% Agar gel.

**Colour and Clarity of prepared medium**
Grey-black coloured, opalescent gel forms in Petri plates

**Reaction**
Reaction of 4% w/v aqueous solution at 25°C. pH : 6.9±0.2

**pH**

6.70-7.10

**Cultural Response**

M892: Cultural characteristics observed on addition of Legionella Supplement(FD041A and FD040) after an incubation at 35-37°C in humid atmosphere.

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of colony |
|---|---|---|---|---|
| **Cultural Response** | | | | |
| *Escherichia coli ATCC 25922* | 50-100 | none-poor | <=10% | |
| *Legionella dumoffii ATCC 33343* | 50-100 | luxuriant | >=50% | light blue-grey |
| *Legionella pneumophila ATCC 33153* | 50-100 | luxuriant | >=50% | white grey to blue grey |
| *Staphylococcus epidermidis ATCC 12228* | 50-100 | none-poor | <=10% | |

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

## Reference

1.Broome C. V., Fraser D. W., 1979, Epidemiol. Rev 1:1-16.
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4.Jones G. T., Hebert G. A., (Eds.), 1979, US Department of Health, Education and Welfare Publication No. (CDC) 79-8375, Atlanta, Centers for Disease Control.
5.Feeley J. C., Gibson R. J., Gorman G. W. et al, 1979, J. Clin. Microbiol., 10:437.
6. Vicker R., Brown and Garrity, 1981, J. Clin. Microbiol., 13:380.
7.Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.
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