

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
#-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 (FD041 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 (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 Petriplates

Reaction

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

Cultural Response

M892: Cultural characteristics observed on addition of Legionella Supplement(FD041 and FD040) after an incubation at 35-37°C in humid atmosphere.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony		
<i>Escherichia coli</i> ATCC 25922	50-100	none-poor	≤10%			

<i>Legionella dumoffii</i> ATCC 33343	50-100	luxuriant	>=50%	light blue-grey		
<i>Legionella pneumophila</i> ATCC 33153	50-100	luxuriant	>=50%	white grey to blue grey		
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	none-poor	<=10%			

Reference

1. Broome C. V., Fraser D. W., 1979, Epidemiol. Rev 1:1-16.
2. George J. R. et al, 1980, J. Clin. Microbiol., 11:286
3. Feeley J. C., Gorman G. W., Weaver R. E. et al, 1978, J. Clin. Microbiol., 8 : 320-325.
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.
8. Bopp C. A., Sumner J. W., Morris G. K. and Wells J. G., 1981, J. Clin. Microbiol., 13:714.

Storage and Shelf Life

Store at 30°C and prepared medium at 2-8°C. Use before expiry period on the label.