



PRODUCT SPECIFICATION SHEET

Legionella Agar Base (DM1072)

Intended Use

Legionella Agar Base (DM1072) is recommended with addition of supplements, used for cultivation of Legionella species.

Product Summary and Explanation

Legionella is a pathogenic group of gram-negative bacteria and is the causative agent of Legionellosis (all illnesses caused by *Legionella*) including a pneumonia type illness called Legionnaires disease and a mild flu like illness called Pontiac fever. Natural sources of *Legionella* are fresh water ponds and creeks. Transmission to humans takes place via inhalation of aerosols from cooling towers, hot water systems or fountains containing the bacteria.

In 1979, Feely et al.⁽¹⁾ described Legionella Agar as a modification of an existing medium, F-G Agar, by replacing starch by charcoal and casein hydrolysate by yeast extract which resulted in better recovery of *Legionella pneumophila*.⁽²⁾ In 1980, Pasculle et al.⁽³⁾ reported that the buffering the medium with addition of ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer improved the nutritive value of the medium. Edelstein⁽⁴⁾ further increased the sensitivity of the medium addition of alpha-ketoglutarate to increase the sensitivity of this medium. Legionella Agar is a modification of the BCYE Agar formula of Edelstein. In the formula, the concentration of ACES buffer was reduced from 10.0 g/L to 6.0 g/L.

Principles of the Procedure

Legionella Agar Base contains yeast extract which provides the necessary nitrogenous nutrients for *Legionella* growth. a-ketoglutarate satisfies the specific nutritional requirements of *Legionella* species. Activated charcoal nullifies toxic compounds that either accumulate in the medium during growth or develop during sterilization of medium. Addition of ACES buffer helps in maintaining proper pH of the medium for optimal growth of *Legionella*. Antibiotics in the supplement inhibit the growth of various contaminating bacteria and fungi.

Formula / Liter

Ingredients	Gms / Liter
Yeast extract	10.00
Charcoal activated	1.50
ACES buffer	6.00
Alpha-Ketoglutarate	1.00
Potassium hydroxide	1.50
Agar	17.00
Final pH: 6.9 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

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

Directions

1. Suspend 18.5 grams in 500 ml distilled water.
2. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
3. Do not heat prior to sterilization.
4. Cool to 50°C and aseptically add rehydrated contents of 1 vial of Legionella Growth Supplement (MS162) or Legionella Supplement (MS150) and Legionella Selective Supplement (MS137).
5. Mix well and pour into sterile Petri plates. Stir the medium during dispensing to prevent settling of charcoal particles.



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Quality Control Specifications

Dehydrated Appearance	Grey to black homogeneous free flowing powder
Prepared Medium	Black coloured opaque gel forms in Petri plates
Reaction of 3.7% Solution	pH : 6.9 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.7% agar gel

Expected Cultural Response: Cultural characteristics observed with added Legionella Growth Supplement (MS162), or Legionella Selective Supplement (MS137) and Legionella supplement (MS150) after an incubation at 35-37°C for 48-72 hours.

Sr. No.	Organisms	Results to be achieved	
		Growth	Colour of Colony
1.	<i>Legionella dumoffii</i> ATCC 33343	good-luxuriant	light blue to grey white
2.	<i>Legionella pneumophila</i> ATCC 33153	good-luxuriant	light blue to grey white

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

Test Procedure

1. Homogenize the specimens in sterile distilled water, for the isolation of *Legionella* species from clinical samples, examine microscopically for *Legionella* by fluorescent antibody (FA) method.
2. Inoculate FA positive cultures on Legionella Agar Base.
3. Incubate the plates at 35°C in 90% relative humidity atmosphere.
4. Growth usually appears in 2-3 days but continue to examine the plates daily for 14 days before discarding them.

Results

Legionella spp. produce small to large, light blue to grey white colonies that fluoresce yellow-green under long-wave UV light.

Storage

Store the 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.

Expiration

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

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Legionella Agar Base

Product Code : DM1072

Available Pack sizes : 100gm



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References

1. Feeley J. C., Gorman G. W., Weaver R. E. Mackel D. G., Smith H. W., 1978, J. Clin. Microbiol., 8(3):320.
2. Feeley J. C., Gibson R. J., Gorman G. W., Langdard N. C., Rasheed J. K., Mackel D.C. and Baine W. B., 1979, J. Clin. Microbiol., 10(4):437.
3. Pasculle A. W., Feeley J. C., Gibson R. J., Cordes L. J., Myerowitz R. L., Patton C. M., Gorman G. W., Cormack C. L., Ezzell J. W., Dowling J. N., 1980, J. Infect. Dis., 141:727.
4. Edelstein P. H., 1981, J. Clin. Microbiol., 14:298.

Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) - 400607. M.S. INDIA.
Ph: +91-9320126789/9833630009/9819991103
Email: sales@micromasterlab.com

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