Legionella Agar Base

Intended Use
Legionella Agar Base with added supplements is used for the cultivation of *Legionella* species.

Summary
Legionella Agar Base is used for isolation of *Legionella* species from clinical and non-clinical specimens. Feely *et al.,* developed Legionella agar as a modification of F-G Agar by replacing the starch with activated charcoal and casein hydrolysate with yeast extract. Pasculle reported that the agar could be improved by buffering the medium with ACES (N-2-acetamido-2-amino ethane sulphonic acid) buffer. Edelstein further increased the sensitivity of the medium by adding α-Ketoglutarate.

Principle
Yeast extract provides nutrients for bacterial growth. α-Ketoglutarate meets the specific nutritional requirements of *Legionella* species. The activated charcoal decomposes hydrogen peroxide, a toxic metabolic product, and may also collect CO_2 and modify surface tension. ACES buffer helps in maintaining proper pH of the medium for the optimal growth of *Legionella* species. Antibiotics in the selective supplement inhibit the growth of various contaminating bacteria and fungi.

Formula*

Ingredients	**g/L**
Yeast Extract	10.0
ACES Buffer	6.0
Potassium Hydroxide	1.5
Charcoal Activated	1.5
α-Ketoglutarate	1.0
Agar	17.0
Final pH (at 25°C)	6.9 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 18.50 g of the powder in 500 mL purified / distilled water and mix well.
2. DO NOT HEAT PRIOR TO STERILIZATION.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Cool to 45°C-50°C and aseptically add rehydrated contents of 1 vial each of Legionella Growth Supplement (204120530010) and Legionella Selective Supplement (204120540005).
5. Mix well and stir the medium while dispensing into sterile petridishes to prevent charcoal particles from settling down.

Quality Control
Dehydrated Appearance: Grey coloured, homogenous, free flowing powder.
Prepared Appearance: Black coloured, opaque gel forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation of 48-72 hours at 30°C-35°C.

Organism (ATCC)	Growth
Legionella pneumophila (33153)	Good

Interpretation of Results

1. Growth is usually visible after 3 days, but may take upto 2 weeks to appear.
2. *L. pneumophila* produce small to large, smooth, colourless to pale blue-grey, slightly mucoid colonies that fluoresce yellow-green when exposed to longwaveUV light.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

1. *Legionella* species are highly pathogenic if inhaled. Handle liquid cultures in a protective cabinet and avoid creating aerosols.
2. Decontaminate working surfaces with 5% hypochlorite solution and autoclave all materials before discarding.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Feely J. C., *et al.,* 1978, J. Clin. Microbiol., 8(3):320.
2. Feely, Gibson, Gorman, *et al.,* 1979, J. Clin. Microbiol., 10(4):437.
3. Pasculle, Feely, Gibson *et al.,* 1980, J. Infect. Dis., 141:727.
4. Edelstein, 1981, J. Clin. Microbiol., 14:298.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201120150100	Dehydrated Culture Media	100 g
201120150500	Dehydrated Culture Media	500 g