



PRODUCT SPECIFICATION SHEET

Asparagine Proline Broth (DM318)

Intended Use

Asparagine Proline Broth (DM318) is recommended for cultivation of *Pseudomonas aeruginosa* from water samples by MF technique.

Product Summary and Explanation

Pseudomonas aeruginosa is one of the most commonly isolated pathogens, and is one of the major contaminants of natural, fresh and recreational water, with the entry being contaminated by wastewater. It is the most frequently isolated non-fermentative bacillus in clinical specimens, infusion fluids, disinfectants and cosmetics. The organism causes disease in humans; e.g., ocular infections, burn wound infections and respiratory tract infections.^(1, 2) This organism is a significant cause of burn and nosocomial infections.⁽³⁾ The ability of *Pseudomonas aeruginosa* to destroy tissue may be related to the production of various extracellular enzymes.⁽²⁾ *Ps. aeruginosa* is an opportunistic pathogen that can multiply in recreational waters in the presence of sufficient nutrients. It produces a water soluble, fluorescent pigment in media containing asparagine and ethanol. Asparagine Proline Broth is recommended for cultivation of *Ps. aeruginosa* by the membrane filter technique. The medium is recommended by BIS.⁽⁴⁾

Principles of the Procedure

Asparagine Proline Broth contains both the enantiomeric forms of Asparagine, which is readily utilized by *Pseudomonas* for their growth. Phosphate and sulphates provide the ions for the growth as well as buffers the medium to promote the growth of the organism.

Formula / Liter

Ingredients	Gms / Liter
DL-Asparagine	2.00
L-Proline	1.00
Dipotassium phosphate, anhydrous	1.00
Magnesium sulphate	0.50
Potassium sulphate	10.00
Final pH: 7.2 ± 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 14.5 grams (for single strength medium) or 29 grams (for concentrated medium) in one liter of distilled water containing 25 ml or 40 ml ethanol respectively.
2. Heat to boiling to dissolve the medium completely.
3. Distribute as desired in screw-capped bottles.
4. Close the caps so that the seal in the lid just touches the lip of the bottle.
5. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
6. Tighten the caps of the bottles immediately after removal from the autoclave to prevent loss of ethanol by evaporation.





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Quality Control Specifications

Dehydrated Appearance	White to cream homogeneous free flowing powder
Prepared Medium	Colourless clear solution, without any precipitate
Reaction of % Solution	Not Applicable
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Escherichia coli ATCC 25922</i>	50-100	none to poor
2.	<i>Pseudomonas aeruginosa ATCC 27853</i>	50-100	luxuriant with greenish yellow pigment

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

Test Procedure

1. When 1 ml of sample is to be analyzed, add 1 ml of sample to 4 ml of single strength medium (14.5 g/l).
2. If larger portions of the sample (10 ml, 50 ml) are to be used, add the sample to an equal volume of the concentrated medium (23.2 g/l).
3. Incubate at 37 ± 1°C for 48 hours. Examine for growth and fluorescence.
4. The growth is further sub cultured on Milk Agar w/ Cetrimide (DM349BS).
5. Refer to appropriate references for standard test procedures.

Results

Refer to appropriate references and standard test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 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. It is not advisable to use polypropylene caps without seals. Alternatively, ethanol may be sterilized separately by filtration and then added aseptically to the sterile cooled medium.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Asparagine Proline Broth

Product Code : DM318





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Available Pack sizes : 100gm / 500gm

References


1. Kiska and Gilligan. 1999. *In* Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Baron, E. J., L. R. Peterson, and S. M. Finegold. 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
3. Gilligan, P. H. 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
4. Bureau of Indian Standards (BIS), 2005, Draft IS 13428:2005.



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