

PRODUCT SPECIFICATION SHEET

Acetamide broth (Twin Pack) (DM445)

Intended Use

Acetamide Broth (Twin Pack) (DM445) is recommended for confirmation of *Pseudomonas aeruginosa* in water samples.

Product Summary and Explanation

A variety of media formulations have been developed to determine the ability of various nonfermenting gram-negative organisms to deaminate acetamide for purposes of identification. Acetamide Broth is formulated as per the recommendation of Standard Methods for the Examination of Water and Wastewater.⁽¹⁾ Assimilation studies by Gilardi and others using basal mineral media showed that acetamide was utilized by a wide variety of nonfermenting organisms.^(1, 2) However very few organisms growing in the medium metabolize acetamide by the process of deamination (acrylamidase activity).^(4, 5) This unique ability is useful in identification of various non-fermenting gram negative organisms.⁽⁶⁻⁸⁾ This ability is shown by *Pseudomonas aeruginosa*, *Pseudomonas aciovorans* Group III (*Achromobacter xylosoxidans*) and *Alcaligenes odorans*.⁽⁹⁾

Principles of the Procedure

Acetamide broth contains inorganic phosphates in the media serve as buffering agents, magnesium sulphate is a source of ions that stimulate metabolism whereas acetamide serves as the sole nitrogen and carbon source. Sodium chloride maintains osmotic equilibrium. Phenol red is the pH indicator.

Formula / Liter

Ingredients	Gms / Liter
Part A	
Acetamide	10.00
Part B	
Sodium chloride	5.00
Dipotassium hydrogen phosphate	1.39
Potassium dihydrogen phosphate	0.73
Phenol red	0.012
Magnesium sulphate	0.50
Final pH: 7.0 ± 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 7.63 grams of part B in one liter of distilled water.
2. Add 10 grams of Part A.
3. Heat to boiling, to dissolve the medium completely.
4. Dispense in tubes or as desired.
5. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Part A :Colourless deliquescent crystals Part B : Light yellow to light pink homogeneous free flowing powder
Prepared Medium	Orange coloured clear solution in tubes
Reaction of solution (Mixture of 1% w/v Part A and 0.76% Part B)	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 4-7 days.

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Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Deamination
1.	<i>Stenotrophomonas maltophilia</i> ATCC 13637	50 -100	good-luxuriant	negative reaction, no purplish red colour within 7days
2.	<i>Pseudomonas aeruginosa</i> ATCC 27853	50 -100	good-luxuriant	positive reaction, purplish red colour within 7 days

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

Test Procedure

1. Inoculate the Acetamide Broth with a loopful of culture emulsified in Casein Soyabean Digest Broth (DM277).
2. Incubate inoculated slant at $35 \pm 2^{\circ}\text{C}$ and observe daily for 4 days and again at 7 days before discarding as negative.
3. Refer appropriate references for specific test procedures.

Results

1. Acetamide deamination leads to the liberation of ammonia, which thereby increases the pH of the medium, leading to a subsequent colour change of the phenol red indicator from yellow orange to purplish red.
2. Complete identification requires determination of the Gram reaction, cellular morphology, biochemical reactions, etc.
3. Refer appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at $10 - 30^{\circ}\text{C}$. 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. Some strains deaminate acetamide slowly and may require as long as 7 days to yield a positive test result.
2. Only about 40% of apycyanogenic strains of *P. aeruginosa* will produce a positive reaction.
3. It is therefore, not advisable to rely on this test as the only criterion for identification.
4. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
5. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Acetamide Broth (Twin Pack)

Product Code : DM445

Available Pack sizes : 100 gm / 500gm

References

1. Gilardi. 1974. Antonie van Leewenhoek. J. Microbiol. Serol. 39:229.
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3. Eaton A. D., Clesceri L. S., and Greenberg A. W., (Eds.), 1995, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
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6. Hedberg, 1969, Appl. Microbiol., 17: 481
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9. Oberhofer and Rowen, 1974, Appl. Microbiol., 28:720.



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Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

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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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