PRODUCT SPECIFICATION SHEET

Acetamide Agar (Twin Pack) (DM444)

Intended Use
Acetamide Agar (DM444) is recommended for differentiation of *Pseudomonas aeruginosa* and other non-fermentative gram-negative bacteria.

Product Summary and Explanation
Acetamide Agar 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.[2, 3] 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 gramnegative organisms.[6, 7, 8] The ability to deaminate acetamide (acylamidase activity) has been found to be most actively accomplished by *P. aeruginosa, Comamonas acidovorans, Achromobacter xylosoxidans* subsp. *xylosoxidans* (*Alcaligenes xylosoxidans*) and *Alcaligenes faecalis (odorans)*.[9] 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. Some strains require upto seven days to exhibit a positive reaction as they deaminate acrylamide slowly. However, only about 40% of apyocyanogenic strains of *Pseudomonas aeruginosa* exhibit a positive reaction. Therefore, it is advisable not to rely on this test as the only criterion for identification.

Principles of the Procedure
Acetamide Agar contains inorganic salts and acetamide a sole carbon and nitrogen source. Sodium chloride maintains the 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
Agar	15.00
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 22.63 grams of part B in 1000 ml distilled water.
2. Add 10.0 grams of Part A. Heat to boiling to dissolve the medium completely.

3. Dispense in tubes or as desired.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool the tubes in a slanted position

Quality Control Specifications

Dehydrated Appearance	Part A :Colourless deliquescent crystals Part B : Light yellow to brick red homogeneous free flowing powder
Prepared Medium	Orange coloured clear to slightly opalescent gel forms in tubes as slants
Reaction of (Mixture of 1% w/v Part A and 2.263% Part B) Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

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

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

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

Test Procedure

1. Inoculate the Acetamide Agar slant with a loopful of culture emulsified in Trypticase Soy Broth.
2. Incubate inoculated slant at 35 ± 2°C and observe daily for 4 days and again at 7 days before discarding as negative.
3. Refer to appropriate references for standard test procedures.

Results

1. Deamination of the acetamide is indicated by a pronounced purplish-red color of the medium.
2. Complete identification requires determination of the Gram reaction, cellular morphology, biochemical reactions, etc.

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. Some strains deaminate acetamide slowly and may require as long as 7 days to yield a positive test result.
2. Only about 37% of apyocyanogenic strains of *P. aeruginosa* will produce a positive reaction. Therefore, this test should not be relied upon as a sole criterion for identification.

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

Packaging
Product Name : Acetamide Agar (Twin Pack)
Product Code : DM444
Available Pack sizes : 500gm
References

1. 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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3. Stainier, Palleroni and Doudoroff, 1966, J. Gen Microbiol., 43:159.
4. Pickett M. J. and Pedersen M.M., 1970, Can. J. Microbiol.,16:351.
5. Pickett M. J. and Pedersen M.M., 1970, Can. J. Microbiol., 16:401.
6. Hedberg, 1969, Appl. Microbiol., 17: 481
7. Smith and Dayton, 1972, Appl. Microbiol., 24: 143
8. Buhlmann, Vischer and Bruhin, 1961, J. Bacteriol., 82:787
9. Oberhofer and Rowen, 1974, Appl. Microbiol., 28:720.

Further Information
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	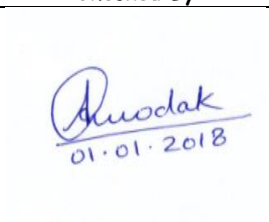	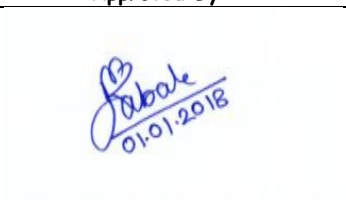
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