Technical Information

Acetamide Broth (Twin Pack)

Product Code: DM 1148

Application:- Acetamide Broth is recommended for confirmation of *Pseudomonas aeruginosa* in water samples

Composition**

Ingredients	Gms / Litre
Part A	-
Acetamide	10.000
Part B	-
Sodium chloride	5.000
Dipotassium hydrogen phosphate	1.390
Potassium dihydrogen phosphate	0.730
Magnesium sulphate	0.500
Phenol red	0.012
Final pH (at 25°C)	7.0±0.2

Principle & Interpretation

Acetamide Broth is formulated as per the recommendation of Standard Methods for the Examination of Water and Wastewater [(1)]. Acetamide is utilized by a wide variety of non-fermenting organisms [(2, 3)]. The media contains inorganic salts and acetamide a sole carbon and nitrogen source. However very few organisms growing in the medium metabolize acetamide by the process of deamination (acrylamidase activity) [(4, 5)]. Acetamide deamination leads to the liberation of ammonia, which thereby increases the pH of the medium, resulting a in subsequent colour change of the phenol red indicator from yellow orange to purplish red. Some strains require upto seven days to produce a positive reaction as they deaminate acrylamide slowly. This unique ability is useful in identification of various non-fermenting gram-negative organisms namely *Pseudomonas aeruginosa , Pseudomonas aciovorans* Group III (*Achromobacter xylosoxidans)* and *Alcaligenes odorans* [(6-9)]. However, only about 40% of apyocyanogenic strains of *Pseudomonas aeruginosa* exhibit a positive reaction. It is therefore, not advisable to rely on this test as the only criterion for identification.

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 used as pH indicator.

Methodology

Suspend 7.63 grams of part B in 1000 ml distilled water. Add 10.0 grams of Part A. Shake well heat if necessary to dissolve the medium completely. Dispense in tubes or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance

Part A : Colourless deliquescent crystals Part B : Light yellow to light pink homogeneous free flowing powder

Colour and Clarity of prepared medium Orange coloured clear solution in tubes

Reaction

Reaction of the medium (Mixture of 1% w/v Part A and 0.76% w/v of Part B) at 25°C. pH : 7.0±0.2

pH Range:- 6.80-7.20

Cultural Response/Characteristics

DM 1148: Cultural characteristics observed after an incubation at 35-37°C for 4-7 days.

Organism	Inoculum (CFU)	Growth	Deamination
Pseudomonas aeruginosa ATCC 27853	50-100	good - luxuriant	positive reaction, purplish red colour (within 7days)
Stenotrophomonas maltophilia ATCC 13637	50-100	good - luxuriant	Negative reaction,no purplish red colour (after 7 days)

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1.Eaton A.D.,Clesceri L.S., and Greenberg A.W., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA, Washington, D.C.
2.Gilardi, 1974, Antonie Van Leeuwenhoek, J. Microbiology Serol., 39:229. 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.

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