# TM 1943 – ACETAMIDE BROTH (DOUBLE PACK) (ISO 16266-2:2018)

## INTENDED USE

For confirmation of non-fermentative Gram-negative bacteria, particularly *Pseudomonas aeruginosa.*

## PRODUCT SUMMARY AND EXPLANATION

A wide variety of pathogenic microorganisms can be transmitted to humans through use of natural fresh and marine recreational waters contaminated by waste water. *Pseudomonas aeruginosa* is one of the organisms that are capable of growth in water at very low concentrations of nutrients. While the primary indicators of water quality are *Escherichia coli* and Enterococci, the enumeration of *Pseudomonas aeruginosa* in recreational waters may be useful in cases of discharge of pulp and paper wastes and effluents from textile finishing plants into receiving waters. One of the unique properties of *P. aeruginosa* is its ability to produce ammonia from acetamide.

Acetamide Broth, formulated as per DRAFT prEN 12780:1999 is recommended for the confirmation of non-fermentative gram-negative *Pseudomonas aeruginosa*. Organisms growing in this medium metabolize acetamide by process of deamination (acrylamidase activity). This ability is shown by Ps. aeruginosa, Ps. acidovorans Group III (Achromobacter xylosoxidans) and *Alcaligens odorans*.

The test water samples are filtered through sterile cellulose ester membrane filters. These filters are aseptically placed on Pseudomonas Agar Base containing Cetrinix Supplement. These plates with filters are incubated at 35- 37°C for 24-48 hours. Pyocyanin-producing colonies are counted as confirmed Ps.aeruginosa . Non-pyocyanin- producing fluorescent colonies are counted as presumptive Ps.aeruginosa . These presumptive Ps.aeruginosa colonies are confirmed by using Acetamide Broth. Production of ammonia from acetamide can be detected by the addition of Nesslers reagent.

## COMPOSITION

| Ingredients | Gms / Ltr |
|---|---|
| **Part I** | |
| **Acetamide** | 2.000 |
| **Part II** | |
| **Sodium chloride** | 0.200 |
| **Potassium dihydrogen phosphate** | 1.000 |
| **Magnesium sulphate anhydrous** | 0.200 |
| **Iron sulphate** | 0.0005 |
| **Sodium molybdate** | 0.005 |

## PRINCIPLE

Acetamide in the medium serves as a sole source of nitrogen and carbon. Magnesium sulphate, sodium molybdate and iron sulphate are the sources of ions that stimulate metabolism. Phosphate serves as a buffering agent.

## INSTRUCTION FOR USE

- Dissolve 1.4 grams of part II in 1000 ml distilled water.
- Add 2 grams of Part I. Heat if necessary to dissolve the medium completely.
- Dispense in tubes or as desired. Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.

## QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder** : Part I : Colourless deliquescent crystals Part II : Off white to white homogeneous free flowing powder

**Appearance of prepared medium** : Colourless clear solution.

**pH (at 25°C)** : 7.0±0.5

## INTERPRETATION

Cultural characteristics observed after incubation.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Deamination | Incubation Temperature | Incubation Period |
|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 27853 | 50-100 | Good-luxuriant | Positive, yellow to brick red colour formation on addition of Nessler's reagent | 35-37°C | 18-24 Hours |
| *Stenotrophomonas maltophilia* | 13637 | 50-100 | Good-luxuriant | Negative, no colour formation on addition of Nessler's reagent | 35-37°C | 18-24 Hours |

## PACKAGING:

In pack size of 100 gm and 500 gm bottles.

## STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

## DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

## REFERENCES

1. Cabelli V. J., 1980, U. S. Environmental Protection Agency, Research Triangle Park, N.C.
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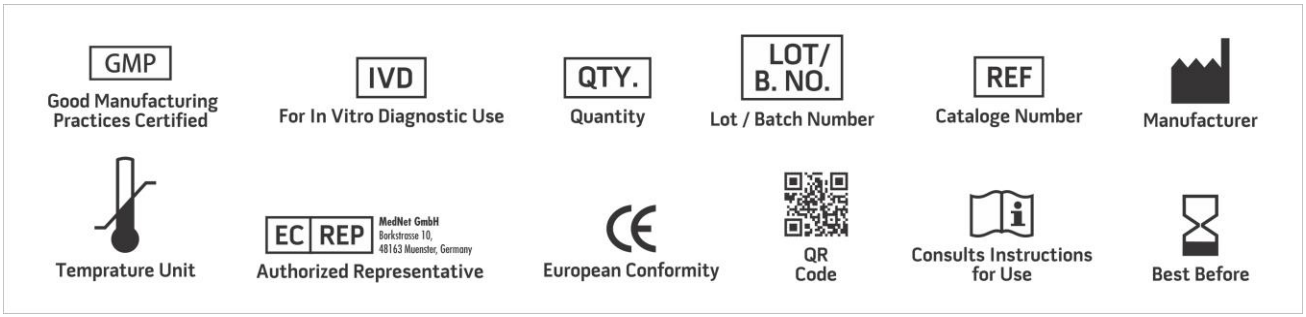
3. Directive of Council of the European Union, Draft prEN 12780:1999
4. Pickett M. J. and Pedersen M. M., 1970, Can. J. Microbiol., 16:351.
5. Pickett M. J. and Pedersen M. M., 1970, Can. J. Microbiol., 16:401.
6. Oberhofer and Rowen, 1974, Appl. Microbiol., 28:720.
7. International Organisation for Standardization(ISO),2006,Draft ISO/DIS,16266.




**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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