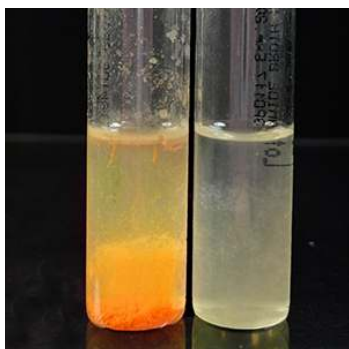


ACETAMIDE BROTH

Dehydrated and ready-to-use culture medium



Positive confirmation of *P.aeruginosa* at left
and negative at right

1 - INTENDED USE

For the confirmation test of *Pseudomonas aeruginosa* according to ISO 16266.

2 - COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Potassium dihydrogen phosphate	1.0 g
Sodium chloride	0.2 g
Magnesium sulphate anhydrous	0.2 g
Acetamide	2.0 g
Sodium molybdate	5.0 mg
Iron sulphate heptahydrate	0.5 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Acetamide Broth contains acetamide as a sole source of carbon for the growth. It is recommended by ISO 16266 for the confirmation of presumptive *Pseudomonas aeruginosa* colonies isolated from water samples by membrane filtration method.¹

Acetamide Broth is based on the ability of non-fermenting Gram-negative bacteria to deaminate the acetamide with production of ammonia which increases the pH of the medium indicated by the change in colour of the Nessler reagent added to the broth.

Potassium dihydrogen phosphate has a high buffering capacity, sodium chloride contributes to the osmotic balance, while magnesium sulphate, sodium molybdate and iron sulphate allow the selective growth of *Pseudomonas*.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 3.4 g in 1000 mL of cold purified water. Adjust the pH to 7.0 ± 0.5 at $25 \text{ }^\circ\text{C}$ with either hydrochloric acid or sodium hydroxide, if necessary. Dispense this mixture in 5 mL aliquots to culture tubes which are then capped and sterilized in an autoclave at $121 \pm 3 \text{ }^\circ\text{C}$ for 15 min.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Prepared tubes appearance	colourless, limpid
Final pH at $20\text{-}25 \text{ }^\circ\text{C}$	7.0 ± 0.5

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Acetamide Broth	Dehydrated medium	40101012	500 g (146.8 L)
Acetamide Broth	Ready-to-use tubes	5510101	20 x 5 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, ancillary culture media and reagents.

8 - SPECIMENS

Acetamide Broth is not intended for primary isolation from samples; it is inoculated with pure colonies from a culture on solid media.

9 - TEST PROCEDURE

Subculture all, or if impracticable, as many as possible of the colonies cultivated on the selective medium requiring confirmation from the membrane filter to Nutrient Agar and incubate for $22 \pm 2 \text{ h}$ at $36 \pm 2 \text{ }^\circ\text{C}$. Check the subcultures for purity and test those that were initially reddish brown for the oxidase reaction.

Inoculate a tube of Acetamide Broth with the subculture and incubate at $36 \pm 2 \text{ }^\circ\text{C}$ for $22 \pm 2 \text{ h}$

10 - READING AND INTERPRETATION

After incubation, add 1 to 2 drops of Nessler reagent and examine the tubes for the production of ammonia, characterized by the production of a colour varying from yellow to brick red depending upon concentration.

Count as confirmed *P. aeruginosa* all colonies which produce pyocyanin (blue/green pigment on Pseudomonas Agar F – King Medium B) or which are oxidase positive, fluoresce under UV radiation and are able to produce ammonia from acetamide.¹

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>P. aeruginosa</i> ATCC 10145	$34\text{-}38^\circ\text{C}$ / $20\text{-}24 \text{ H}$ / A	growth with colour change of Nessler Reagent
<i>E. coli</i> ATCC 11775	$34\text{-}38^\circ\text{C}$ / $20\text{-}24 \text{ H}$ / A	no growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection





12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated and ready-to-use Acetamide Broth is tested for specific growth characteristics by comparing the results with a previously approved Reference Batch. The medium is tested with target strains inoculated in pure culture: *P. aeruginosa* ATCC 9027, *P. aeruginosa* ATCC 10299, *P. aeruginosa* ATCC 14207. After incubation at 37°C for 24 hours, all target strains exhibit good growth in the liquid medium and the Nessler Reagent added to the tubes turns to orange-red colour. The medium is tested with non-target strains inoculated in pure culture *P. putida* ATCC 49128 and *E. coli* ATCC 11775. Both the non-target strains develop a negative test with the Nessler Reagent.

13 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated Acetamide Broth is classified as dangerous because contains acetamide, a carcinogenic and irritant compound. Before use, consult the Material Safety Data Sheets.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving.
- Each tube of this culture medium is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging). According to ISO 16266, the self-prepared tubes can be stored at +2°C +8°C in the dark for up to 3 months.

Ready-to-use tubes

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

15 - REFERENCES

1. ISO 16266:2006 Water quality – Detection and enumeration of *Pseudomonas aeruginosa* by membrane filtration.

TABLE OF APPLICABLE SYMBOLS

or Catalogue number	Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

