Acetamide Medium
Art. No. 03-428

Specification
Liquid test medium use for the confirmation of Pseudomonas aeruginosa in water according to the ISO 16266:2006 standard.

Formula* in g/L

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetamide</td>
<td>2,0000</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>0,2000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0,2000</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>0,0005</td>
</tr>
<tr>
<td>Mono-potassium phosphate</td>
<td>1,0000</td>
</tr>
<tr>
<td>Sodium molybdate</td>
<td>0,0050</td>
</tr>
</tbody>
</table>

Final pH 7,0 ± 0,5 at 25ºC

Directions
Dissolve 3,4 g of powder in 1 L of distilled water. Heat only if necessary. Distribute in screw-capped tubes and sterilize in the autoclave at 121ºC for 15 minutes. Prepared medium may be opalescent and with precipitate. To obtain a clear transparent medium without precipitation, avoid heating and sterilize the medium by filtration. The sterilized medium (with or without precipitates) remains active for 3 months if it is stored in the dark in a cool place.

Description
This nutrient solution uses acetamide as the sole carbon and nitrogen source, and therefore it only allows the growth of those microorganisms that are able to use acetamide. In water and in almost all food stuffs, these microorganisms are the non fermenting Gram negative bacillus, Pseudomonas aeruginosa is the only organism that can liberate ammonia by deamination of acetamide. Some authors suggest the use of this nutrient solution as an enrichment medium prior to the use of isolation medium, will reduce false positives from heavily polluted samples. Comamonas acidovorans, Achromobacter xylosidans and Alcaligenes faecalis (odorans) can also deaminate acetamide, but can not growth on the plating medium that is selective for Pseudomonas.

Technique
To confirm potential Pseudomonas aeruginosa colonies on the Cetrimide Agar (Art. No. 01-160 or 01-609) they must first be cultured on a non-selective medium to obtain pure cultures from which perform the confirmation tests. Acetamide Medium is inoculated with a couple of colonies from the pure culture and is incubated at 36 ± 2ºC for 22 ± 2 hours. Add 2 drops of Nessler’s 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 of ammonia present.

References
- DIN Standard 3841. Deutsche Einheitsverfahren zür Wasser, Abwasser und Schlammuntersuchung Mikrobiologische Verfahren: Nachweis von Pseudomonas aeruginosa (K8).

Storage
For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4ºC to 30ºC and <60% RH).

Quality control

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Growth</th>
<th>Remarks</th>
</tr>
</thead>
</table>
| Pseudomonas aeruginosa ATCC 27853 | Good | Green pigment. Acet (+)
| Pseudomonas aeruginosa ATCC 15442 | Good | Green pigment. Acet (+)
| Pseudomonas aeruginosa ATCC 9027 | Good | Green pigment (48 h). Acet (+)
| Escherichia coli ATCC 25922   | Inhibited | -

Left: Pseudomonas aeruginosa ATCC 27853
Centre: Pseudomonas aeruginosa ATCC 9027
Right: Uninoculated tube