Nutrient Agar ISO

Cat. 1156

For the isolation of a pure culture for the confirmation of Pseudomonas aeruginosa.

Practical information

Aplications	Categories
Non selective enumeration	Pseudomonas aeruginosa

Industry: Water

Regulations: ISO 16266

Principles and uses

Nutirent Agar is a medium used to obtain a pure culture for the confirmation of the presumptive positive colonies obtained in Pseudomonas CN Agar Base (Cat. 1153).

Pseudomonas aeruginosa is an opportunist pathogen for humans, capable of growing in water with a low concentration of nutrients. This is why natural mineral water and spring water are Pseudomonas aeruginosa free at the time of their commercialization. This microorganism can also be found in swimming pool water.

Peptone and beef extracts provide the nitrogen, vitamins, minerals and amino acids nutrient source. Yeast extract is a vitamins source, particularly of the B-group, essential for bacterial growth. Sodium chloride maintains the osmotic balance and the bacteriological agar is the solidifying agent.

Formula in g/L

Bacteriological agar	15	Beef extract	1
Peptone	5	Sodium chloride	5
Yeast extract	2		

Preparation

Suspend 28 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 118 ºC for 15 minutes. Cool to 45-50 ºC, mix well and dispense into plates.

Instructions for use

According to ISO 16266 for the detection and enumeration of Pseudomonas aeruginosa:
- Filter a certain volume of water sample through a filter membrane and place the membrane on a Pseudomonas CN Agar Base plate (Cat. 1153).
- Incubate at a temperature of 36±2 °C for 44±4 h.
- Count the colonies that have a green/blue pigmentation (pyocyanin) as confirmed P. aeruginosa.
- Examine the membrane under UV light.
- All colonies that are fluorescence (+) and reddish-brown colonies should be confirmed.
- Spread all the colonies that should be confirmed on Nutrient Agar (Cat. 1156) to obtain pure cultures. Incubate at 36±2 ºC for 22±2 h
- Perform oxidase assay to the reddish-brown colonies.
- Streak the oxidase (+) colonies on King B Medium (Cat. 1154) to check the fluorescence production. Incubate at 36±2 ºC for up to 5 days. Normally 24 hours are enough.
- Inoculate all the fluorescence (+) colonies, both in CN agar and in King B Medium, in the Acetamide Broth (Cat. 1155 o Cat. 2017) medium and add one or two drops of Nessler reagent to check the ammonia production. Incubate at 36±2 ºC for 22±2 h.
- The colonies that produce pyocyanin in CN agar, the colonies fluorescence (+) in CN Agar and ammonia (+) in Acetamide Broth, and the reddish brown colonies in CN Agar, oxidase (+), fluorescence (+) in King B Agar and ammonia (+) in Acetamide Broth, are counted as confirmed P. aeruginosa.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber, slightly opalescent	7,4±0,2

Microbiological test

Incubation conditions: (36±2 ºC / 22±2 h)
Inoculation conditions: Productivity qualitative (10^3-10^4 CFU)

Microrganisms	Specification
Escherichia coli ATCC 25922	Good growth
Pseudomonas aeruginosa ATCC 27853	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

UNE-EN 12780 Quality of water. Identification and enumeration of Pseudomonas aeruginosa by membrane filtration.
EN ISO 16266 Water quality -- Detection and enumeration of Pseudomonas aeruginosa -- Method by membrane filtration