King A Medium (Pseudomonas P Agar) ISO

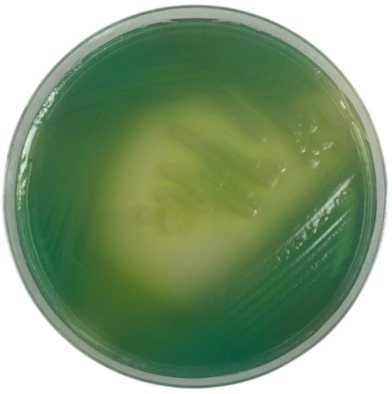
Cat. 1531

For the identification and confirmation of Pseudomonas spp based on pyocyanin production.

Practical information

Aplications	Categories
Confirmation	Pseudomonas aeruginosa
Detection	Pseudomonas

Industry: Water



Principles and uses

King A Medium (Pseudomonas P Agar) is prepared according to the formula described by King et al. for the detection and differentiation of Pseudomonas aeruginosa from other Pseudomonas based on pyocyanin production.

Pseudomonas aeruginosa is a free-living bacterium, present in soil and water. It has become more and more known as an emerging opportunistic pathogen of clinical importance. Various different epidemiological studies track its occurrence as a nosocomial pathogen and claim that antibiotic resistance is increasing in clinical isolates.

This medium promotes the production of pyocyanin, a blue-green pigment which oxidizes to brown, is water-soluble and, unlike fluorescein, is soluble in chloroform. The pigment diffuses throughout the medium and the blue color is observed. Confirmation of pyocyanin production is by chloroform extraction. Add 2 ml of chloroform to a tube of medium and shake gently to remove pigment.

This medium contains pancreatic digest of gelatin as a rich nitrogen source, and other nutrients for growth as vitamins, minerals and amino acids. Gelatin peptone is low in phosphorous to reduce the inhibitory action on pyocyanin production. Potassium sulfate and Magnesium chloride provide cations to activate pyocyanin production and enhance pigment production. Glycerol is a carbon source. Bacteriological agar is the solidifying agent.

Formula in g/L

Bacteriological agar	15	Gelatin pancreatic digest	20
Potassium sulfate	10	Magnesium chloride	1,4

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 46,4 grams of the medium in one liter of distilled water. Add 10 ml of glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121 ºC for 15 minutes.

Instructions for use

For the confirmation of Pseudomonas aeruginosa according to ISO 22717:
- Subcultivate the presuntive colonies of Pseudomonas aeruginosa (yellow-green pigment and fluorescence under UV radiation) obtained in Cetrimide Agar (Cat. 1102).
- Incubate the plates at 32,5±2,5 ºC for 24,48 and 72 h.
- Pseudomonas aeruginosa form colonies surrounded by a blue to green zone due to pyocyanin formation or with a red to dark brown zone due to pyorubin production.

Quality control

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

Microbiological test

Incubation conditions: (32,5±2 ºC / 24-48-72 h).

Microorganisms	Specification	Characteristic reaction
Pseudomonas aeruginosa ATCC 10145	Good growth	Pyocyanin production (blue-green colonies)
Burkholderia Cepacia ATCC 25608	Good growth	No pyocyanin production
Pseudomonas aeruginosa ATCC 27853	Good growth	Pyocyanin production (blue-green colonies)
Pseudomonas fluorescens ATCC 49838	Good growth	No pyocyanin production
Pseudomonas aeruginosa ATCC 9027	Good growth	Pyocyanin production (blue-green colonies)

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

King E.O. Ward M.K. Raney D.E.-J. Lab. and Clin Med, 1954. 44. 301-307
Bacteriological Analytical Manual, 8th edition. 1995. AOAC International, Gaithersburg, MD.
The United States Pharmacopoeia. 1995. The United States Pharmacopoeia, 23rd ed. United States Pharmacopoeial Convention, Rockville, MD.