

King A Medium (Pseudomonas P Agar) ISO

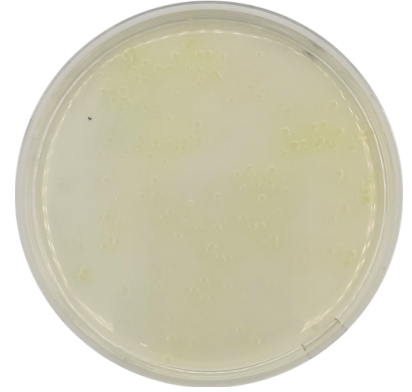
Cat. 1531

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

Practical information

| Applications | Categories |
|--------------|-------------------------------|
| Confirmation | <i>Pseudomonas aeruginosa</i> |
| Detection | <i>Pseudomonas</i> |

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 °C 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 °C 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 | Appearance | Color of the dehydrated medium | Color of the prepared medium | Final pH (25°C) |
|------------|-------------|--------------------------------|------------------------------|-----------------|
| w/o rests | Fine powder | Light beige | Amber, slightly opalescent | 7,2±0,2 |

Microbiological test

Incubation conditions: (32,5±2 °C / 18-24 h).

| Microorganisms | Specification | Characteristic reaction |
|--|---------------|--|
| <i>Pseudomonas aeruginosa</i> ATCC 10145 | Good growth | Pyocyanin production (blue-green colonies) |
| <i>Escherichia coli</i> ATCC 25922 | Good growth | No pyocyanin production |
| <i>Pseudomonas aeruginosa</i> ATCC 27853 | Good growth | Pyocyanin production (blue-green colonies) |
| <i>Escherichia coli</i> ATCC 8739 | Good growth | No pyocyanin production |
| <i>Pseudomonas aeruginosa</i> ATCC 9027 | Good growth | Pyocyanin production (blue-green colonies) |

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

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.