



# KING A MEDIUM (PSEUDOMONAS P AGAR) USP

CAT Nº: 1531

For the identification of *Pseudomonas spp* based on pyocyanin production

## FORMULA IN g/l

Final pH 7.0 + 0.2 at 25°C					
Potassium Sulfate	10.00	Bacteriological Agar	13.60		
Gelatin Pancreatic Digest	20.00	Magnesium Chloride	1.40		

## PREPARATION

Suspend 45 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. The prepared medium should be stored at 8-15°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

#### 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 and fluorescein (pyoverdin) inhibition.

*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 contains Gelatin pancreatic digest 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.

Inoculate and incubate at  $35 \pm 2^{\circ}$ C for 18 - 24 hours.

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.

## **MICROBIOLOGICAL TEST**

The following results were obtained in the performance of the medium from type cultures, with glycerol added, after incubation at a temperature of  $35 \pm 2^{\circ}$ C and observed after 18 - 24 hours.

Microorganisms	Growth	Colony Color
Pseudomonas aeruginosa ATCC 9027	Good	Blue
Pseudomonas aeruginosa ATCC 25619	Good	Blue-green
Pseudomonas aeruginosa ATCC 27853	Good	Blue



## **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.



# STORAGE

Once opened keep powdered medium closed to avoid hydration.

