Technical Data

King's Broth B Base

M5262

Kings Broth B Base is recommended for non-selective cultivation and pigment production of *Pseudomonas* species

Composition**

Ingredients	Gms / Litre
Proteose peptone No.3	20.000
Dipotassium hydrogen phosphate	1.500
Magnesium sulphate. heptahydrate	1.500
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22.23 grams(the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water containing 15 ml of glycerol. Heat if necessary to dissolve the medium completely. Mix well and dispense in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Pseudomonas aeruginosa is known to produce two types of pigments, pyocyanin and fluorescein useful in isolation and detection of *Pseudomonas* species from various specimen samples such as clinical,water,food,cosmetics etc. An additional pigment called as pyorubin was reported by King et al (1).Some strains of *Pseudomonas* produce all the three pigments while the others produce one or two pigments.Pyocyanin is green while fluorescein is fluorescent yellow and pyorubin is reddish brown.

P.aeruginosa can be identified on Hugh Leifson Medium (M826). Kings Broth B base is based on the formulation of King et al (1, 2),except the agar and can be used as a general medium for the non-selective cultivation and pigment production of *Pseudomonas* species .Kings Broth B base is particularly suited for production and detection of fluorescein (pyoverdin), a yellowish green pigment that fluoresces under ultrviolet light.

The Broth contain proteose peptone, which provides Both carbonaceous and nitrogenous compounds for the growth of bacteria. Glycerol serves as a source of energy and also enhances pigment production. Magnesium sulphate also enhances pigment production. Pigments and/ or their derivatives produced by *Pseudomonas* species plays a role as siderophores in the iron uptake systems of bacteria, and hence, their production is markedly enhanced under conditions of iron deficiency. The addition of dipotassium phosphate increases the phosphorus content of the medium thereby enhancing production of fluorescent pigment, while inhibiting the production of pyocyanin.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured, clear to slightly opalescent solution in tubes

Reaction
Reaction of 2.22% w/v aqueous solution (containing 1.5%v/v glycerol) at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Pigment production

Cultural Response

Pseudomonas aeruginosa ATCC 17934	50-100	good-luxuriant	greenish yellow
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	greenish yellow
Pseudomonas aeruginosa ATCC 9027	50-100	good-luxuriant	greenish yellow
Burkholderia cepacia ATCC 25609	50-100	good-luxuriant	no pigment

Storage and Shelf Life

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1.King E. O., Ward M. K. and Raney D. E., 1954, J. Lab and Clin. Med., 44:301-307.
2.Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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