Technical Information

King's medium B Base

Product Code: DM 2544

Application: - Kings Medium B Base is recommended for non-selective isolation, cultivation and pigment production of *Pseudomonas* species.

Composition**

Ingredients	Gms / Litre
Proteose peptone	20.000
Dipotassium hydrogen phosphate	1.500
Magnesium sulphate. heptahydrate	1.500
Agar	20.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Kings Medium B Base is based on the formulation of King et al (1, 2). It can be used as a general medium for the non-selective isolation and pigment production of *Pseudomonas* species from foods, cosmetic samples etc. *Agrobacterium* have been traditionally identified as gram-negative bacteria that do not produce fluorescent pigment on Kings B medium and do produce tumors (or hairy roots) when inoculated onto test plants (3).

Pseudomonas aeruginosa is known to produce two types of pigments, pyocyanin and fluorescein which is a characteristic property and aids in isolation of *Pseudomonas* from clinical material. An additional pigment called as pyorubin was reported by King. Pyocyanin is green while fluorescein is fluorescent yellow and pyorubin is reddish brown. Some strains produce all these pigments while the others produce one or two pigments. *P.aeruginosa* can be identified on Hugh Leifson Medium (DM1826). Kings Medium B Base is particularly suited for fluorescein.

Proteose peptone, which supply carbon and nitrogen compounds for the bacterial metabolism. Glycerol act as a source of energy and also serves as a pigment production. Magnesium sulphate also enhances pigment production. Pigments and/ or their derivatives produced by *Pseudomonas* species play a role as siderophores in the iron uptake systems of bacteria, and hence, their production is markedly enhanced under conditions of iron deficiency. The production of pigments especially non-fluorescent blue pigment, pyocyanin is readily demonstrated by culturing on Kings Medium B, which contains no added iron (4). The addition of dipotassium phosphate increases the phosphorus content of the medium thereby enhancing production of fluorescent pigment. For inoculation, use the organisms freshly cultured in Kings Medium A, incubate overnight at 37°C and then at room temperature for 6 days. With Kings Medium B, incubate at 37°C for 6 days.

Methodology

Suspend 42.23 grams of dehydrated powder media in 1000 ml distilled water containing 15 ml of glycerol. Mix thoroughly & heat to boiling to dissolve the medium completely. Mix well. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Aseptically pour into sterile Petri plates.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% Agar gel

Colour and Clarity
Light yellow coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.22% w/v aqueous solution (containing 1.5%v/v glycerol) at 25°C. pH : 7.2±0.2

pH Range
7.00-7.40

Cultural Response
DM1544: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Pigment production
Pseudomonas aeruginosa ATCC 17934	50-100	good-luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 9027	50-100	good-luxuriant	>=70%	greenish yellow
Burkholderia cepacia ATCC 25609	50-100	good-luxuriant	>=70%	no pigment

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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.
3. Ann G., Matthysse, 1998, The Genus Agraobacterium, Chapter 3.1.4. Martin Dworkin, 3rd Ed., The Prokaryotes, An Evolving Electronic Resource for the Micrbiological Community.
4. Todar K., Todars Online Textbook of Bacteriology, University of Wisconsin - Madison, Department of Bacteriology.

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