King's Medium B Base

M1544I

Intended Use:

Recommended for detection and enumeration of *Pseudomonas aeruginosa* by membrane filtration. The composition and performance criteria of this medium are as per the specifications laid down in ISO 16266:2006(E)

Composition**

ISO 16266:2006(E)Specification -King's Medium B Base

Ingredients	g / L
Peptone	20.000
Dipotassium hydrogen phosphate	1.500
Magnesium sulphate, heptahydrate	1.500
Agar	15.000
Final pH (at 25°C)	7.2±0.2

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**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.23 grams (the equivalent weight of dehydrated medium per litre) of dehydrated medium in 1000 ml purified / distilled water containing 10 ml of glycerol. Heat to boiling to dissolve the medium completely. Mix well. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically pour into sterile Petri plates.

Principle And Interpretation

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 (M826). Kings Medium B Base is particularly suited for fluorescein.

Kings Medium B Base is based on the formulation of King et al (1,2). This medium 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).King's Medium B Base is recommended by ISO Committee (4). These media contain proteose peptone, which provides 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 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 (5). 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.

Type of specimen

Water samples.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(6) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Results should be noted after 18-24 hours. Else it might result in erroneous results.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 3.72% w/v aqueous solution (containing 1.0%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.

Organism	Inoculum (CFU)	Growth	Recovery	Pigment production
Pseudomonas aeruginosa ATCC 17934	50-100	good-luxuriant	>=70%	greenish yellow
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=70%	greenish yellow
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50-100	good-luxuriant	>=70%	greenish yellow
Burkholderia cepacia ATCC 25609	50-100	good-luxuriant	>=70%	no pigment

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

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4.Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane filtration- International Organization for Standardization-ISO 16266:2006(E).

5.Todar K., Todars Online Textbook of Bacteriology, University of Wisconsin - Madison, Department of Bacteriology.

6.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

7.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition

8.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. .1

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