King's Medium B Base w/ 1.5% Agar

M1544F

Intended Use:

Recommended for non-selective isolation, cultivation and pigment production of *Pseudomonas* species in accordance with FDA BAM, 2017.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.0 grams 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. 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 its isolation from from clinical and food samples. An additional pigment entitled pyorubin was reported by King(5). Pyocyanin is green, fluorescein is fluorescent yellow and pyorubin is reddish brown in colour. Some strains produce all the three pigments while the others produce one or two. Kings Medium B Base w/ 1.5% agar, recommended by FDA BAM is particularly suited for fluorescein production (2). This medium can be used as a general medium for the non-selective isolation and pigment production of *Pseudomonas* species from foods, cosmetics etc (1). This media contain proteose peptone, which provides carbonaceous and nitrogenous compounds for the growth of bacteria. Glycerol serves as a source of energy and also as an enhancer in 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 Base w/ 1.5% Agar, which contains no added iron (7). The addition of dipotassium phosphate increases the phosphorus content of the medium thereby enhancing production of fluorescent pigment. The media can also be dispensed in tubes as slants and butt.

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (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. 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 3.8% 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 aeruginosa 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 (3,4).

Reference

1. Ann, G, and Matthysse. 1998. The Genus Agraobacterium. The Prokaryotes 3 ed.
2. FDA, U.S. 2017. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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
5. King, E. O, M. K Ward, and D. E Raney. 1954. J. Lab and Clin. Med 44: 301-307.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Todar K., Todars Online Textbook of Bacteriology, University of Wisconsin -Madison, Department of Bacteriology.

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