PRODUCT SPECIFICATION SHEET

King's Medium B Base (DM1392)

Intended Use

King's Medium B Base (DM1392) is recommended for non-selective isolation, cultivation and pigment production of *Pseudomonas* species.

Product Summary and Explanation

Pseudomonas species is a free-living, environmental organism found in water and soil and on plants, including fruits and vegetables. It is a gram-negative, aerobic, coccobacillus bacterium with unipolar motility. It is an opportunistic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants. *P. aeruginosa* secretes a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown) which is an additional pigment reported by King. Pyocyanin and fluorescein is a characteristic property and aids in isolation of *Pseudomonas* from clinical material. Some strains produce all these pigments while the others produce one or two pigments. *P. aeruginosa* can be identified on Hugh Leifson Medium (DM370). 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. 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. Pigment production especially non-fluorescent blue pigment, pyocyanin is readily demonstrated by culturing on Kings Medium B, which contains no added iron.[4] *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]

Principles of the Procedure

King's Medium B Base contains proteose peptone, which provides carbon, nitrogen and other essential nutrients required for the growth of bacteria. Glycerol serves as a source of energy and also enhances pigment production. Magnesium chloride, potassium sulphate also enhances pigment production. Production of fluorescent pigment is enhanced by the addition of dipotassium phosphate increases the phosphorus content of the medium thereby enhancing.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	20.00
Dipotassium hydrogen phosphate	1.50
Magnesium sulphate. heptahydrate	1.50
Agar	20.00
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 42.23 grams of the medium in one litre of distilled water containing 15 ml of glycerol.
2. Heat to boiling to dissolve the media completely. Mix well.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Aseptically pour into sterile Petri plates.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 4.22% solution (containing 1.5%v/v glycerol)	pH 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 2.0% Agar gel

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

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Pigment production
1.	*Pseudomonas aeruginosa ATCC 17934*	50-100	good-luxuriant	>=70%	greenish yellow
2.	*Pseudomonas aeruginosa ATCC 27853*	50-100	good-luxuriant	>=70%	greenish yellow
3.	*Pseudomonas aeruginosa ATCC 9027*	50-100	good-luxuriant	>=70%	greenish yellow
4.	*Burkholderia cepacia ATCC 25609*	50-100	good-luxuriant	>=70%	no pigment

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

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. Refer appropriate references for standard test procedures.

Results

Refer appropriate references and procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : King's Medium B Base
Product Code : DM1392
Available Pack sizes : 500gm

References

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. Todar K., Todars Online Textbook of Bacteriology, University of Wisconsin - Madison, Department of Bacteriology.
4. 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.

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Further Information

For further information please contact your local MICROMASTER Representative.

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