AuSaMicS™ Life Science

TDS

TDS-AS-1260

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King's B Medium Base

Pseudomonas Agar F / Flo Agar | Catalog No. AS-1260

1. Product Identity

Product Name	King's B Medium Base
Catalog Number	AS-1260
Synonyms	Pseudomonas Agar F, Flo Agar, King's Medium B, Fluorescein Agar, Pigment Production Agar B
Medium Type	Differential, dehydrated (peptone-based)
Weighed Quantity	38.0 g/L + 10–15 mL/L glycerol (added separately)
Final pH (25°C)	7.2 ± 0.2
Appearance (prepared)	Light amber, clear

2. Overview

King's B Medium Base is a specialised differential medium designed to enhance the production of fluorescein (pyoverdin) — a diffusible, water-soluble, yellow-green pigment that fluoresces brightly under long-wave UV light (366 nm). It is a critical tool for the presumptive identification of Pseudomonas species in clinical, environmental, and pharmaceutical samples.

The medium is formulated with phosphorus-rich peptone and magnesium sulfate, both of which activate fluorescein synthesis while suppressing pyocyanin formation — the complementary pigment targeted by the related King's A Medium.

3. Principle & Mode of Action

Fluorescein (pyoverdin) is a siderophore synthesised by fluorescent pseudomonads under iron-limited conditions to scavenge environmental iron. King's B Medium promotes this biosynthetic pathway by supplying abundant phosphorus and magnesium while limiting readily available iron, making pigment expression — and therefore UV fluorescence — a reliable phenotypic marker. Because Pseudomonas fluorescens and Pseudomonas putida also fluoresce on this medium, a positive result is presumptive and should be confirmed with oxidase testing, growth at 42°C, and/or biochemical or molecular confirmation for definitive P. aeruginosa identification.

4. Historical Development

King's B Medium was first described by Elizabeth O. King, M.K. Ward, and D.E. Raney in their 1954 paper, “Two simple media for the demonstration of pyocyanin and fluorescin,” published in the Journal of Laboratory and Clinical Medicine. The paper introduced two complementary formulations — King A for pyocyanin and King B for fluorescein — that remain the reference standard for pigment-based Pseudomonas differentiation more than seven decades later. The formulation is also codified by the U.S. FDA as BAM Media M128 for fluorescein production testing.

5. Typical Applications

- Differentiation of Pseudomonas aeruginosa from other non-fermenting Gram-negative bacilli
- Presumptive confirmation of Pseudomonas isolates from wound, respiratory, and urinary specimens
- Supportive use in USP/EP microbial limit testing workflows for detection of P. aeruginosa
- Environmental monitoring in pharmaceutical and cosmetic manufacturing
- Detection and enumeration of fluorescent pseudomonads in water samples (ISO 16266-aligned workflows)

6. Nutrient Formulation (per Litre)

Ingredient	Concentration	Function
Peptone (mixed)	20.0 g	Amino acids, nitrogen, carbon; high phosphorus for pigment induction
Dipotassium phosphate	1.5 g	Phosphorus source for pigment expression; buffering
Magnesium sulfate	1.5 g	Cation cofactor activating fluorescein synthesis
Bacteriological agar	15.0 g	Solidifying agent
Glycerol (added separately)	10–15 mL	Carbon and energy source

Glycerol is not included in the dehydrated base powder and must be added during preparation. For reference, the FDA BAM M128 formulation specifies Proteose Peptone No. 3 (20 g), Tryptone (10 g), dipotassium phosphate (1.5 g), magnesium sulfate (0.73 g), glycerol (10 mL), and agar (15 g) per litre — a closely related published standard.

7. Preparation

- Suspend 38.0 g of dehydrated medium in 1 L of purified water. Add 10–15 mL glycerol
- Heat to boiling with agitation to dissolve the agar completely
- Sterilise by autoclaving at 121°C for 15 minutes
- Cool to 45°C and pour into sterile plates. Store prepared plates at 2–8°C

8. Quality Control (Fluorescence / Growth Promotion Testing)

Test Organism	Inoculum	Expected Result (24–48 h, 25–30°C)
Pseudomonas aeruginosa ATCC 27853	≤ 100 CFU	Good growth; bright yellow-green fluorescence under UV (366 nm)
Pseudomonas fluorescens ATCC 13525	≤ 100 CFU	Good growth; fluorescence expected (non-specific for species ID)
Escherichia coli ATCC 25922	≤ 100 CFU	Growth permitted; no fluorescence (differential check)
Staphylococcus aureus ATCC 25923	≤ 100 CFU	Growth permitted to inhibited; no fluorescence

9. Storage & Stability

- Dehydrated medium: store at 10–30°C, dry, protected from moisture
- Prepared medium: store at 2–8°C, protected from light
- Typical shelf life: refer to batch-specific Certificate of Analysis

10. Intended Use

For laboratory, research, and quality-control use only. Not for direct administration to humans or animals. A positive fluorescence result is presumptive and requires confirmatory testing for definitive species identification.

11. Literature / References

- King, E.O., Ward, M.K., & Raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. Journal of Laboratory and Clinical Medicine, 44(2), 301–307.
- U.S. Food and Drug Administration. Bacteriological Analytical Manual (BAM), Media M128 — Pseudomonas Agar F (for fluorescein production).
- International Organization for Standardization. ISO 16266 — Water quality: detection and enumeration of Pseudomonas aeruginosa.