TECHNICAL DATA SHEET

AS-1259

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King Agar A

King's Medium A | Pseudomonas Agar P | Pyocyanin Detection Medium

Document Control	Version 1.0 · Reviewed 31 August 2025
Technical Review	Hassan Salimi, Technical Director, AuSaMicS Pty Ltd (18+ years culture media formulation)

Product Identification

Product Name	King Agar A
Synonyms	King's Medium A; Pseudomonas Agar P
Catalogue Number	AS-1259
Format	Dehydrated powder (glycerol added separately)
Manufacturer	AuSaMicS Pty Ltd, Melbourne, Australia

Intended Use

Differential medium for confirmation of Pseudomonas aeruginosa by demonstration of pyocyanin (blue-green, non-fluorescent, diffusible pigment) in clinical, water, food, and environmental microbiology.

Mode of Action

Gelatin peptone supplies nitrogen, carbon, sulfur, and trace elements while being deliberately low in phosphate, since excess phosphate inhibits pyocyanin biosynthesis. Potassium sulfate and magnesium chloride are the specific activating cations required for pyocyanin production. Glycerol, added as a separate liquid supplement, serves as the carbon source supporting pigment expression. The medium is differential rather than selective: essentially any organism able to grow will do so, but only pyocyanin producers (chiefly P. aeruginosa) generate the diagnostic diffusible blue-green zone.

> *Formulation note: this medium correctly contains magnesium chloride (MgCl2), consistent with the original King, Ward & Raney (1954) formula and current BD Difco / Sigma-Aldrich / HiMedia technical documentation. Magnesium sulfate is a component of King B medium (fluorescein detection), not King A.*

Formulation (per litre)

Ingredient	Quantity	Function
Gelatin Peptone (pancreatic digest)	20.0 g	Nitrogen/carbon source, low phosphate
Potassium Sulfate (K2SO4)	10.0 g	Pyocyanin-activating cation
Magnesium Chloride (MgCl2, anhydrous)	1.4 g	Pyocyanin-activating cation
Agar	15.0 g	Solidifying agent

Glycerol (added separately)	10.0 mL	Carbon/energy source for pigment expression

Total dehydrated components: 46.4 g/L (excluding glycerol). **Final pH: 7.2 ± 0.2 at 25°C**

Preparation

- Suspend 46.4 g of dehydrated medium in 990 mL purified water
- Add 10 mL glycerol
- Heat with frequent agitation and boil for 1 minute to dissolve completely
- Sterilise by autoclaving at 121°C for 15 minutes
- Cool to 45–50°C, mix well, and pour plates

Incubation Conditions

Parameter	Condition
Temperature	35 ± 2°C
Time	18–24 hours (routine); reincubate at 25–30°C for 24–48h if pigment weak
Atmosphere	Aerobic
Reading	Daylight; no UV required

Quality Control — Cultural Response

Organism	ATCC No.	Inoculum (CFU/plate)	Expected Result
Pseudomonas aeruginosa	27853	≤ 100	Good growth; strong blue-green pyocyanin pigment
Pseudomonas fluorescens	13525	≤ 100	Growth without pyocyanin (differential control)
Escherichia coli	25922	≤ 100	Growth may occur without characteristic pigment (negative control)

King Agar A vs King Agar B

Property	King Agar A	King Agar B
Target pigment	Pyocyanin (blue-green, non-fluorescent)	Fluorescein / pyoverdine (yellow-green, UV-fluorescent)
Key cation	Magnesium chloride + potassium sulfate	Magnesium sulfate + phosphate buffer
Reading method	Daylight — no UV needed	UV light (365 nm) enhances detection

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Storage & Shelf Life

Dehydrated medium	Below 30°C, tightly closed, protected from moisture/light — 24 months
Prepared plates	2–8°C, protected from light — use within 4 weeks

Limitations & Technical Considerations

- Not strongly selective — use a selective medium (e.g., Cetrimide Agar) for primary isolation from mixed flora before confirming on King Agar A.
- Not all pseudomonads produce pyocyanin — a negative result does not exclude other Pseudomonas species.
- Pigment intensity varies with time — extending incubation can improve detection of weak producers.
- Presumptive, not definitive — interpret alongside oxidase reaction and other confirmatory tests.

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.
- Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A., Yolken, R.H. (Eds.) (2003). Manual of Clinical Microbiology, 8th Ed. ASM Press, Washington DC.
- MacFaddin, J.F. (1985). Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1. Williams & Wilkins, Baltimore.