TECHNICAL DATA SHEET

KING B AGAR

CONFIRMATION OF *PSEUDOMONAS AERUGINOSA*

1 INTENDED USE

King B agar allows the production of fluoresceine (or pyoverdin), a yellow-green pigment that fluoresces under ultraviolet light in certain strains of *Pseudomonas*. The medium is used primarily in water analysis for the detection and differentiation of *Pseudomonas aeruginosa*, which produce the characteristic pigment while other species of *Pseudomonas* do not.

The typical composition responds to that defined in the standard NF EN ISO 16266.

2 HISTORY

The media was described by King, Ward and Raney in 1954, and then modified according to the recommendations of the US Pharmacopoeia. The authors were also responsible for the development of King A medium, which favors production of pyocyanin (a fluorescent blue pigment) over that of pyoverdin.

3 PRINCIPLES

Dipotassium phosphate increases the concentration of phosphorus contributed by the peptone and stimulates the production of fluoresceine, while inhibiting the production of pyocyanin.

Magnesium sulfate contributes the cations necessary for the production of pyoverdin.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media :

- Peptone ..... 20,0 g
- Glycerol ..... 10,0 mL
- Dipotassium phosphate ..... 1,5 g
- Magnesium sulfate, 7 H_2O ..... 1,5 g
- Bacteriological agar ..... 15,0 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

5 PREPARATION

- Melt the media for the minimum amount of time necessary in order to achieve complete liquefaction, then incline the tubes in order to obtain oblique slants after solidification and cooling.
- It is recommended, when the medium has not been used for at least 8 days following its preparation and initial solidification, to regenerate it in a boiling water bath and re-solidify in the proper position.

6 INSTRUCTIONS FOR USE

- Inoculate the inclined surface of King B agar in tight streaks with each suspected colony taken from selective isolation media and previously purified on Nutrient agar.
- Incubate at 36 ± 2 °C for 24 hours to 5 days. Examine daily the culture under UV light and note any eventual fluorescence.

✓ **Inoculation :**
Surface inoculation

✓ **Incubation :**
24 h to 5 days at 36 °C

Note : It is necessary to use pure cultures taken from the center of isolated colonies in order to avoid cross-reactions that render the identification impossible to achieve.

7 RESULTS

Colonies that present a yellow-green fluorescence under UV light at 360 nm are considered positive for *Pseudomonas aeruginosa* for water control according to the method described in the standard NF EN ISO 16266.

8 QUALITY CONTROL

Complete agar : amber agar.

Typical culture response after 24 hours incubation at 36 °C :

Microorganisms		Growth	Fluorescence at 360 nm
Pseudomonas aeruginosa	WDCM 00024	Positive	Positive
Pseudomonas aeruginosa	WDCM 00025	Positive	Positive
Pseudomonas aeruginosa	WDCM 00026	Positive	Positive
Escherichia coli	WDCM 00090	Positive	Negative
Staphylococcus aureus	WDCM 00035	Positive	Negative

9 STORAGE / SHELF LIFE

Ready-to-melt media in tubes : 2-25 °C.
The expiration date is indicated on the label.

10 PACKAGING

Ready-to-melt (complete) media (with glycerol) :
7 x 7 mL tubes BM10508

11 BIBLIOGRAPHY

King, E. O., Ward, M. K., and Raney, D .E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**: 301.

NF EN ISO 16266. Août 2008. Qualité de l'eau. Détection et dénombrement de *Pseudomonas aeruginosa*. Méthode par filtration sur membrane.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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