60786 King Agar B
(Pseudomonas Agar; F Agar; Pseudomonas Agar (for Fluorescein))

For the detection and enumeration of fluorescing bacteria in water, in particular *Pseudomonas fluorescens* in drinking water acc. to King et al. (1954).

Composition:

Ingredients	Grams/Litre
Mixed peptone	20.0
Dipotassium hydrogen phosphate	1.5
Magnesium sulfate	1.5
Agar	10.0

Final pH 7.2 +/- 0.2 (at 25°C)

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions :
Dissolve 33 g in 990 ml distilled water and add 10 ml glycerol (Cat. No 49769). Sterilize by autoclaving at 121°C for 15 minutes.
Optional CFC Selective Supplement (Cat. No 53477) can be added to make the media selective.
According to the Dansk Standard, prepare dilution series of the sample material (dilution factor 10).
Pipet two 1 ml aliquots from each dilution step and inoculate the plate using pour plate method.
Incubate the plates up to 72 hours at 20-25 °C.
Determine the count of the fluorescing bacteria under the UV lamp and the total microbial count.

Principle and Interpretation:
King Agar B enhances the elaboration of fluorescein and inhibits the pyocyanin formation. Mixed peptone provide the essential nitrogenous nutrients, carbon, sulphur and trace elements. Glycerol serves as a
C-source and Dipotassium hydrogen phosphate buffers the medium. Magnesium sulfate is necessary for the activation of fluorescein production [8].
Ps. aeruginosa build colonies surrounded by a yellow to greenish-yellow zone due to fluorescein production which fluoresces under UV light. If pyocyanin is also synthesized, a bright green colour is produced. For the confirmation of pyocyanin the coloured pigments can be extracted with chloroform. Most pyocyanin-producing Pseudomonas strain synthesize also fluorescein and others produce just one pigments. The temperature can be a determinig factor as most fluorescent strains will not grow at 35°C. Rather, they grow 25-35°C. Atypical pyocyanin-negative, fluorescein-positive *Ps. aeruginosa* strains can also be differentiated from *Ps. fluorescens* and *Ps. putida* (6). *Ps. aeruginosa* can be differentiated by following cultivation on Mac Conkey Agar (Cat. No. 70143, because *Ps. putida* and *Ps. fluorescence* do not fluorescens under UV light and grow poorly [7].
CFC Selective Supplement is an antibiotic supplement for the selective isolation of *Pseudomonas* species.

Cultural characteristics after up to 72 hours at 20-25°C.

Organisms (ATCC)	Growth	Yellow-green pigment in daylight	Fluorescens e at 366 nm
Pseudomonas fluorescens (17397)	+++	+	+
Pseudomonas aeruginosa (27853)	+++	+	+
Pseudomonas aeruginosa (9027)	+++	+	+
Pseudomonas aeruginosa (17397)	+++ (48 h)	+/-	+/-
Pseudomonas cepacia (25609)	+++	-	-
Aeromonas hydrophila (7966)	+++	-	-
Escherichia coli (25922)	+++	-	-
Enterobacter cloacae (13047)	+++	-	-

References:

1. E.O. King, et al., Media for the demonstration of pyocyanin and fluorescein, J. Lab. Clin. Med. 44, 301 (1954)
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3. G.J. Bonde, øresunds-Vandkomiteens undersøgelser, 288 (1965-70).
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5. L.C.D Formiga, New possibilies for the laboratory diagnosis of diphtheria, Brazilian J. Med. Biol. Res., 18, 401 (1985)
6. D.J. Blazevic, M.H. Köpcke, J.M. Matsen, Incidence and identification of Pseudomonas fluorescens and Pseudomonas putida in the clinical laboratory, Appl. Microbiol., 25, 107 (1973)
7. M.H. Brodsky, M.C. Nixon, Rapid method for detection of Pseudomonas aeruginosa on Mac Conkey-Agar under ultraviolet light. - Appl. Microbiol., 26, 219 (1973)
8. MacFaddin, Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md. (1985.)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.