

KING B MEDIUM UNE-EN 12780, EN ISO 16266

CAT Nº: 1154

For the identification and enumeration of *Pseudomonas aeruginosa* by membrane filtration

FORMULA IN g/l

Peptone	20.00	Heptahydrated Magnesium Sulfate	1.50
Potassium Hydrogen Phosphate	1.50	Bacteriological Agar	15.00

Final pH 7.2± 0.2 at 25°C

PREPARATION

Suspend 38 grams of the medium in one liter of distilled water. Add 10 ml of glycerol. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes, 5 ml in each tube, and sterilize in autoclave at 121°C for 15 minutes. Allow to cool in a slanted position. The prepared medium should be stored at 2-8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

KING B MEDIUM is prepared according to the UNE-EN 12780. It is a confirmation medium for the detection and differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* based on fluorescein (pyoverdin) production and pyocyanin inhibition.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. It also aids in the production of fluorescein. Potassium hydrogen phosphate is a phosphorus source, and Magnesium sulfate provides cations to activate fluorescein production. Glycerol is a carbon source. Bacteriological agar is the solidifying agent.

Red color colonies from Nutrient Agar (UNE-EN 12780; Cat. 1156) that have are oxidase-positive are sown again and incubated for five days maximum (normally 24 hours are enough) at 36 ± 2°C. The growth is examined daily under ultraviolet light and the presence of any fluorescence is registered.

This medium promotes the production of pyoverdin, a yellow-green fluorescent pigment that can be oxidized to yellow. It is water soluble and, unlike pyocyanin, is not soluble in chloroform. The pigment diffuses throughout the medium and the fluorescent yellow-green color is observed by use of a Wood's UV lamp.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures, with glycerol added, after incubation at a temperature of 36 ± 2°C and observed after 24 hours.

Microorganisms	Growth	Fluorescein
<i>Pseudomonas aeruginosa</i> ATCC 9027	Good	+
<i>Pseudomonas aeruginosa</i> ATCC10145	Good	+
<i>Pseudomonas aeruginosa</i> ATCC25783	Good	+

BIBLIOGRAPHY

UNE-EN 1 2780, Quality of water. Identification and enumeration of *Pseudomonas aeruginosa* by membrane filtration



STORAGE

Once opened keep powdered medium closed to avoid hydration.

