

60788 King Agar A (Pseudomonas Agar for Pyocyanin; Pseudomonas Agar P; Tech Agar)

Medium for the confirmation of *Pseudomonas aeruginosa* by pyocyanin formation.

Composition:

| Ingredients | Grams/Litre |
|--------------------------------|-------------|
| Gelatine peptone (pancreatic) | 20.0 |
| Magnesium chloride | 1.4 |
| Potassium sulfate | 10.0 |
| Agar | 15.0 |
| 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.

Appearance: Slightly beige colored, homogeneous, free flowing powder.

Gelling: Firm

Color and Clarity: Slightly brownish-yellow colored, clear to slightly opalescent gel forms in petri plates.

Directions:

Dissolve 41.4 g in 990 ml distilled water and add 10 ml glycerol (Cat. No.49767). Sterilize by autoclaving at 121°C for 15 minutes.

Principle and Interpretation:

King Agar A enhances the elaboration of phycocyanin and inhibits the fluorescein formation.

Phycocyanin is a blue, water- and chloroform-soluble, nonfluorescent pigment that diffuses into the surrounding medium.

Gelatine peptone provide the essential nitrogenous nutrients, carbon, sulphur and trace elements and is low in phosphorous to minimize the inhibitory action on pyocyanin production. Glycerol serves as a C-source. Potassium sulfate and magnesium chloride is necessary for the activation of phycocyanin production [12].

Ps. aeruginosa build colonies surrounded by a blue-green zone due to phycocyanin production.

Although this medium enhances especially the phycocyanin production (blue pigment), it is possible that red (pyorubin), yellow-green (pyoverdine, fluorescein) or brown (pyomelanine) pigments are produced and mask the phycocyanin. For the confirmation of pyocyanin the coloured pigments can be extracted with chloroform. 0.5-1 ml chloroform is added on a culture slant and it is shaken for a few minutes until the phycocyanin is diffused, which makes the solvent blue. After that some drops of HCl is added and a rapid color change from blue to red appears, this confirms the presence of phycocyanin.

Incubate plates or tubes at 35 ± 2°C for 18-24 hours. If there is no or little growth, reincubate at 25-30°C for 1-2 days and observe for growth and pigment production [8].



Cultural characteristics after up to 24-48 hours at 30 ± 2°C.

| Organisms (ATCC) | Growth | pigments |
|--|--------|--------------|
| <i>Pseudomonas fluorescens</i> (49838) | +++ | - |
| <i>Pseudomonas aeruginosa</i> (27853) | +++ | green-bluish |
| <i>Pseudomonas aeruginosa</i> (25668) | +++ | green-bluish |
| <i>Pseudomonas aeruginosa</i> (9027) | +++ | green-bluish |
| <i>Pseudomonas aeruginosa</i> (10145) | +++ | dark green |
| <i>Pseudomonas cepacia</i> (25609) | +++ | - |
| <i>Escherichia coli</i> (25922) | +++ | - |
| <i>Enterobacter cloacae</i> (13047) | +++ | - |

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