Also known as

Pigment Production Agar A; Ps Medium A; PsP;Tech Agar; King A Medium; Pseudomonas Agar Medium for detection of Pyocyanin.

Specification

Solid medium to enhance the pyocyanin production by *Pseudomonas aeruginosa* according to ISO standards.

Formula * in g/L

Peptone..... 20.0
Magnesium chloride..... 1.4
Potassium sulfate..... 10.0
Agar..... 15.0

Final pH 7,2 ±0,2 at 25 ºC
* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 46,4 g of powder in 1 L of distilled water with glycerol 10 mL and let soak. Heat, stirring constantly, until boiling. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes. If tubes are used, let them solidify with short slant and good sized butt.

Description

This A medium was formulated by King, Ward and Raney in 1954 to enhance the pyocyanin production by *Pseudomonas aeruginosa*. The blue pigment, Pyocyanin, diffuses into the culture medium. Pyocyanin production varies depending on the strains of *Pseudomonas aeruginosa* present and on the growth conditions. Although this medium enhances blue pigment production in particular, it is possible that green (pyoverdine) or brown (pyomelanin) pigments also appear and mask the pyocyanin. Production of fluorescein and other pseudomonas pigments can be observed on other more suitable media, like King B Agar (Art. No. DC1029).

Technique

Slanted tubes or Petri dishes are inoculated by streaking and are then incubated à 30-35°C for 24-48-72h. The disadvantage of using Petri plates is that the medium is subject to dehydration during incubation. Therefore, it is better to use slanted tubes being careful to aerate by loosening the screw caps or replacing them with cotton or aluminium caps.
In freshly isolated pathogenic strains from the pathological material, pigment production is often shown early i.e. after 24 -48- 72 hours of incubation, however if the material is non pathogenic or if it comes from water, food or soil, then the pigmentation can be delayed.
When the pigment is not the usual blue colour, it may be due to the production of two or more coloured substances. If it is not confirmed on other culture medium, it is recommended to confirm by extraction:
Using the culture slant, 0,5-1 mL chloroform is added, and is shaken for a few minutes until the pyocyanin is diffused, which turns the solvent blue. After that, the chloroform is acidified with a few drops of HCl, obtaining a rapid change in colour from blue to red, this colour changes confirms the presence of pyocyanin.

Quality control

Incubation temperature: 30-35ºC **Incubation time:** 24-48-72 h

Inoculum: Pure culture is inoculated by surface streaking

Microorganism	Growth	Remarks
Pseudomonas fluorescens ATCC® 49838	Good to very good	Without pigments. F (-)
Pseudomonas aeruginosa ATCC® 10145	Good to very good	Dark green
Pseudomonas aeruginosa ATCC® 27853	Good to very good	Green to blue
Pseudomonas aeruginosa ATCC® 9027	Good to very good	Green to blue
Burkholderia cepacia ATCC® 25608	Good to very good	Without pigment

References

. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· ISO 22717 Standard (2015) Cosmetics - Microbiology - Detection of Pseudomonas aeuruginosa.
· KING E.O., M. WARD & D.E. RANEY (1954) Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab.Clin.Med. 44:301-307.
· LENNETTE, E.H., E.W. SPAULDING & J.P. TROUANT (1974) Manual of Clinical Microbiology. 2nd. Ed. ASM. Washington.
· USP (2008) 31th ed. <61> Microbial Limit Tests. US Pharmacopeial Convention Inc. Rockville MD.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).