Pseudomonas Agars
Pseudomonas Agar F • Flo Agar
Pseudomonas Agar P • Tech Agar

Intended Use

Pseudomonas Agar F, also known as Flo Agar, is used for the enhancement of fluorescin production and Pseudomonas Agar P, also known as Tech Agar, is used for the enhancement of pyocyanin production by *Pseudomonas*.

Summary and Explanation

Pseudomonas aeruginosa is widely distributed in soil, water and foods. It is frequently isolated from infusion fluids, disinfectants and cosmetics. The organism causes disease in humans; e.g., ocular infections, burn wound infections and respiratory tract infections.[1]

Most strains of *P. aeruginosa* produce pyocyanin, a blue, water- and chloroform-soluble, nonfluorescent pigment that diffuses into the surrounding medium.[2] *P. aeruginosa* is the only *Pseudomonas* species known to produce this pigment. (However, certain strains are apyocyanogenic.)

Some strains of *P. aeruginosa* produce other pigments, such as the brown-black pyomelanin, the red pyorubin or the yellow pyoverdin. Pyoverdin is a water soluble fluorescent pigment often produced by *P. aeruginosa* and other pseudomonads isolated from humans.[2] The presence of these pigments can, however, mask the production of pyocyanin.[2]

Pseudomonas Agar F (Flo Agar) and Pseudomonas Agar P (Tech Agar) are modifications of two media (Medium A and Medium B) that King et al. developed to enhance pigment production for improved differentiation of pseudomonads.[3]

Principles of the Procedure

The 1:1 ratio of casein to meat peptone in Pseudomonas Agar F (Flo Agar) is conducive to fluorescin production by *Pseudomonas*. These peptones contain phosphorus, which is stimulatory to fluorescin production.[4] The addition of dipotassium phosphate increases the phosphorus content of the medium, thereby enhancing production of the fluorescent pigment. Magnesium sulfate provides essential ions for fluorescin production.[4]

Pseudomonas Agar P (Tech Agar) contains enzymatic digest of gelatin to provide amino acids and other essential nitrogenous substances. The gelatin peptone is low in phosphorous to minimize the inhibitory action on pyocyanin production.[4] Magnesium, potassium and sulfate ions promote pyocyanin production.[4]

Both media contain glycerol, which acts as a source of energy and enhances pigment production.

Formulae

Difco™ Pseudomonas Agar F

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Proteose Peptone No. 3	10.0	g
Dipotassium Phosphate	1.5	g
Magnesium Sulfate	1.5	g
Agar	15.0	g

Difco™ Pseudomonas Agar P

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	20.0	g
Magnesium Chloride	1.4	g
Potassium Sulfate	10.0	g
Agar	15.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water containing 10 g of glycerol:
   Difco™ Pseudomonas Agar F – 38 g;
   Difco™ Pseudomonas Agar P – 46.4 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Specimens must first be isolated in pure culture on an appropriate medium. The isolate should be Gram-stained and examined to confirm that morphology is appropriate for *Pseudomonas*.

User Quality Control

Identity Specifications

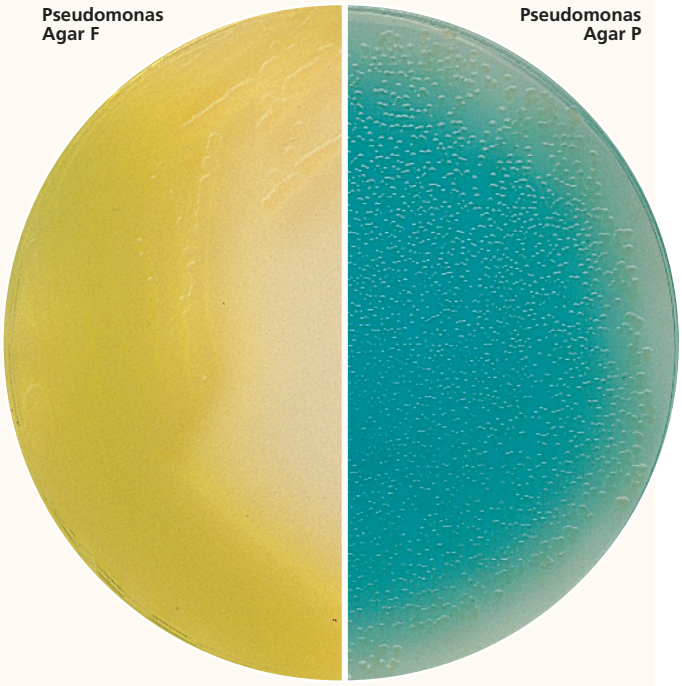
Difco™ Pseudomonas Agar F

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water with 1% glycerol upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 3.8%Solution with 1% glycerol at 25°C:	pH 7.0 ± 0.2

Difco™ Pseudomonas Agar P

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.64% solution, soluble in purified water with 1% glycerol upon boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.64% Solution with 1% glycerol at 25°C:	pH 7.0 ± 0.2

Pseudomonas aeruginosa ATCC™ 9027



Cultural Response

Difco™ Pseudomonas Agar F or Pseudomonas Agar P

Prepare the medium per label directions. For Pseudomonas Agar F inoculate as described below and incubate at 35 ± 2°C for 18-24 hours. For Pseudomonas Agar P inoculate with fresh cultures and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU PSEUDOMONAS AGAR F	RECOVERY	PIGMENT PRODUCTION PSEUDOMONAS AGAR F	PIGMENT PRODUCTION PSEUDOMONAS AGAR P
Pseudomonas aeruginosa	9027	30-300	Good	Greenish yellow	Blue
Pseudomonas aeruginosa	27853	30-300	Good	Greenish yellow	Blue to green
Pseudomonas cepacia	25609	30-300	Good	No pigment	No pigment

Using a sterile inoculating loop or needle, streak plates or slants with several colonies from the subculture medium. Incubate plates or tubes, with caps loosened, at 35 ± 2°C for 18-24 hours. If the isolate fails to grow or grows slowly, reincubate at 25-30°C for 1-2 days and observe for growth and pigment production.[5]

Expected Results

Examine Pseudomonas Agar F (Flo Agar) under long wavelength UV light (366 nm) for fluorescin, a greenish-yellow fluorescent pigment in the colonies and surrounding medium. Examine Pseudomonas Agar P (Tech Agar) for pyocyanin, a blue to blue-green pigment seen in the colonies and surrounding medium. Confirm the presence of pyocyanin by adding several drops of chloroform and observe for a blue color in the chloroform. (Pyocyanin is more soluble in chloroform than in water.)

Limitations of the Procedure

1. Occasionally, a *Pseudomonas* culture is encountered that will produce small amounts of pigment in the medium. When this happens, a yellow-green color will appear on Pseudomonas Agar F (Flo Agar) or a blue-green color on Pseudomonas Agar P (Tech Agar). If a blue-green color occurs on Pseudomonas Agar P (Tech Agar), confirmation of the presence of pyocyanin can be made by extraction with chloroform ($CHCl_3$).[4]
2. The formation of nonpigmented colonies does not completely rule-out *a Pseudomonas aeruginosa* isolate.
3. A pyocyanin-producing *Pseudomonas* strain will usually also produce fluorescin. It must, therefore, be differentiated from other simple fluorescent pseudomonads by other means. Temperature can be a determining factor as most other fluorescent strains will not grow at 35°C. Rather, they grow at 25-30°C.[4]

References

1. Kiska and Gilligan. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
3. King, Ward and Raney. 1954. J. Lab. Clin. Microbiol. *44*:301.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
5. Sewell. 1987. *In* Wentworth (ed.), Diagnostic procedures for bacterial infections, 7th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Pseudomonas Agar F

BAM CCAM

Cat. No. 244820 Dehydrated – 500 g

BBL™ Flo Agar

BAM CCAM

Cat. No. 296003 Prepared Slants – Pkg. of 10

Difco™ Pseudomonas Agar P

BAM

Cat. No. 244910 Dehydrated – 500 g

Europe
Cat. No. 257018 Prepared Plates – Pkg. of 20*

BBL™ Tech Agar

BAM

Cat. No. 296004 Prepared Slants – Pkg. of 10

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

**Store at 2-8°C.*