

## Availability

### Difco™ Proteose No. 3 Agar

Cat. No. 265100 Dehydrated – 500 g

### BBL™ Hemoglobin Solution 2%

Cat. No. 211874 Bottle – 10 × 100 mL\*

### BBL™ Hemoglobin, Bovine, Freeze-Dried

Cat. No. 212392 Dehydrated – 500 g

### Difco™ Supplement B

Cat. No. 227610 Lyophilized – 6 × 10 mL with Reconstituting Fluid\*  
227620 Lyophilized – 100 mL with Reconstituting Fluid\*

### Difco™ Supplement VX

Cat. No. 233541 Lyophilized – 6 × 10 mL with Reconstituting Fluid\*  
233542 Lyophilized – 100 mL with Reconstituting Fluid\*

\*Store at 2-8°C.

# Pseudomonas Agars

## Pseudomonas Agar F • Flo Agar

## Pseudomonas Agar P • Tech Agar

### Intended Use

These media conform with specifications of *The United States Pharmacopeia (USP)*.

*Pseudomonas* Agar F, also known as Flo Agar, is used for the enhancement of fluorescein 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.<sup>1</sup>

Most strains of *P. aeruginosa* produce pyocyanin, a blue, water- and chloroform-soluble, nonfluorescent pigment that diffuses into the surrounding medium.<sup>2</sup> *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 pyromelanin, the red pyrorubin or the yellow pyoverdine. Pyoverdine is a water soluble fluorescent pigment often produced by *P. aeruginosa* and other pseudomonads isolated from humans.<sup>2</sup> The presence of these pigments can, however, mask the production of pyocyanin.<sup>2</sup>

*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.<sup>3</sup>

Both media are listed in the *USP* for use in the performance of Microbial Limit Tests.<sup>4</sup>

### Principles of the Procedure

The 1:1 ratio of casein to meat peptone in *Pseudomonas* Agar F (Flo Agar) is conducive to fluorescein production by *Pseudomonas*. These peptones contain phosphorus, which is stimulatory to fluorescein production.<sup>5</sup> 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 fluorescein production.<sup>5</sup>

*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.<sup>5</sup> Magnesium, potassium and sulfate ions promote pyocyanin production.<sup>5</sup>

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

- 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.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

## User Quality Control

### Identity Specifications

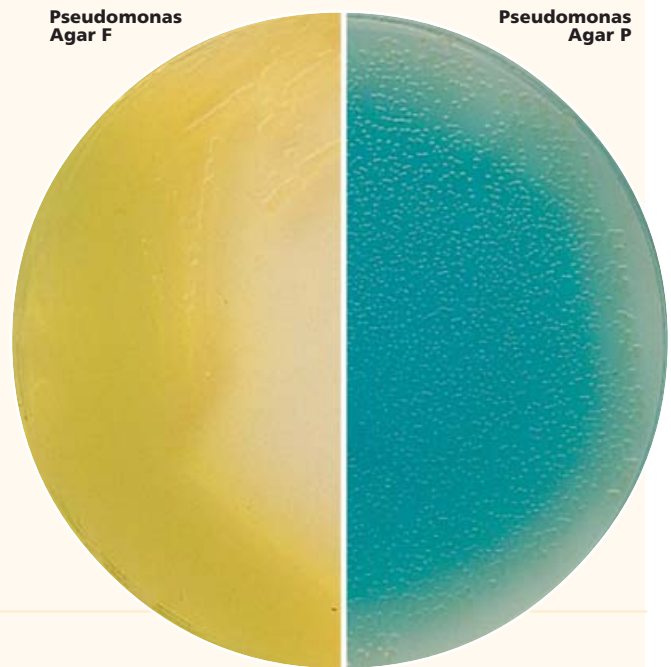
#### Difco™ Pseudomonas Agar F

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. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

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

## 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*.

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.<sup>6</sup>

## Expected Results

Examine Pseudomonas Agar F (Flo Agar) under short wavelength UV light (254 nm) for fluorescein, 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<sub>3</sub>).<sup>5</sup>
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 fluorescein. 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.<sup>5</sup>

## References

1. Kiska and Gilligan. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Forbes, Sahn and Weissfeld. 1998. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo.
3. King, Ward and Raney. 1954. J. Lab. Clin. Microbiol. 44:301.
4. United States Pharmacopeial Convention, Inc. 2001. The U.S. pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
6. 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 USP

Cat. No. 244820 Dehydrated – 500 g

### BBL™ Flo Agar

BAM USP

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

### Difco™ Pseudomonas Agar P

BAM USP

Cat. No. 244910 Dehydrated – 500 g

### BBL™ Tech Agar

BAM USP

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

### Difco™ Glycerol

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

# Pseudomonas Isolation Agar

## Intended Use

Pseudomonas Isolation Agar is used with added glycerol in isolating *Pseudomonas* and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on pigment formation.

## Summary and Explanation

*Pseudomonas aeruginosa* is an opportunistic pathogen that can infect eyes, ears, burns and wounds.<sup>1</sup> It is also a leading cause of hospital acquired infections. Patients undergoing antibiotic therapy are especially susceptible to infection by *Pseudomonas aeruginosa*.

Pseudomonas Isolation Agar is prepared according to a slight modification of the Medium A formulation of King, Ward and Raney.<sup>2</sup> Pseudomonas Isolation Agar includes Irgasan™, a

potent broad spectrum antimicrobial that is not active against *Pseudomonas*.<sup>3</sup> As well as being selective, Pseudomonas Isolation Agar is formulated to enhance the formation of the blue or blue-green pyocyanin pigment by *Pseudomonas aeruginosa*. The pigment diffuses into the medium surrounding growth.

*Irgasan™* is a trademark of Ciba-Geigy.

## Principles of the Procedure

Peptone provides the carbon and nitrogen necessary for bacterial growth. Magnesium chloride and potassium sulfate promote production of pyocyanin. Irgasan, an antimicrobial agent, selectively inhibits gram-positive and gram-negative bacteria other than *Pseudomonas* spp. Agar is the solidifying agent. Glycerol serves as an energy source and also helps to promote pyocyanin production.

## User Quality Control

### Identity Specifications

#### Difco™ Pseudomonas Isolation Agar

Dehydrated Appearance:	Very light beige, homogeneous, free-flowing.
Solution:	4.5% solution, soluble in purified water containing 2% glycerol upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 4.5% Solution at 25°C:	pH 7.0 ± 0.2

### Cultural Response

#### Difco™ Pseudomonas Isolation Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	APPEARANCE
<i>Escherichia coli</i>	25922	10 <sup>3</sup> -2 × 10 <sup>3</sup>	Marked to complete inhibition	–
<i>Pseudomonas aeruginosa</i>	10145	10 <sup>2</sup> -10 <sup>3</sup>	Good	Green to blue-green
<i>Pseudomonas aeruginosa</i>	27853	10 <sup>2</sup> -10 <sup>3</sup>	Good	Green to blue-green

Uninoculated Plate

*Pseudomonas aeruginosa*  
ATCC™ 27853