

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.¹ 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.² *Pseudomonas* Isolation Agar includes Irgasan™, a potent broad spectrum antimicrobial that is not active against *Pseudomonas*.³ 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.

Formula

Difco™ *Pseudomonas* Isolation Agar

Approximate Formula* Per Liter

Peptone	20.0	g
Magnesium Chloride.....	1.4	g
Potassium Sulfate.....	10.0	g
Irgasan™	25.0	mg
Agar	13.6	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water containing 20 mL of glycerol. 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.

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

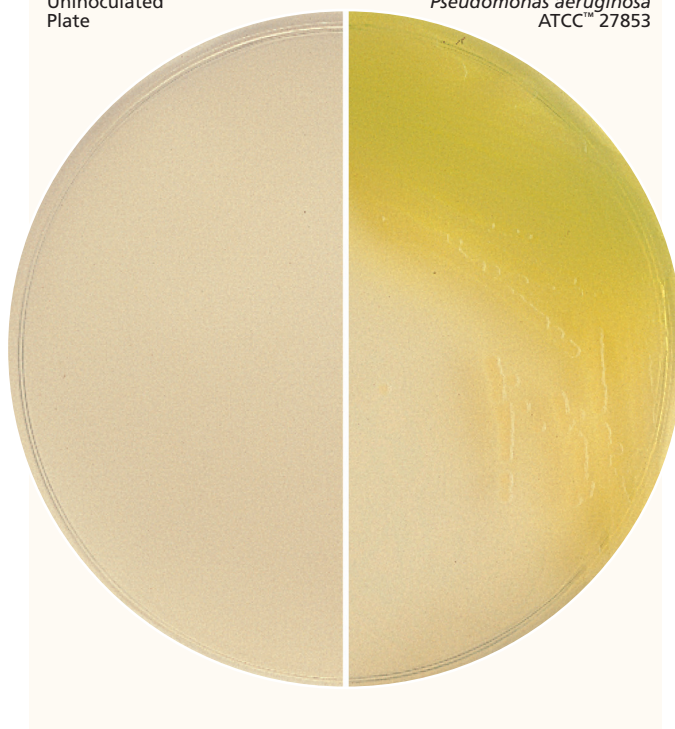
Difco™ *Pseudomonas* Isolation Agar

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 ³ -2 × 10 ³	Marked to complete inhibition	—
<i>Pseudomonas aeruginosa</i>	10145	10 ² -10 ³	Good	Green to blue-green
<i>Pseudomonas aeruginosa</i>	27853	10 ² -10 ³	Good	Green to blue-green

Uninoculated Plate

Pseudomonas aeruginosa
ATCC™ 27853



Procedure

Inoculate the medium using the streak plate method to obtain isolated colonies. Incubate for 18-48 hours at $35 \pm 2^\circ\text{C}$.

Expected Results

Examine for the presence of good growth. *Pseudomonas aeruginosa* colonies may be greenish after incubation for 18 hours and turn blue to blue-green as incubation continues up to 24-48 hours, with diffusion of the pigment into the medium.

Limitations of the Procedure

1. Some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.^{1,4}
2. Non-*Pseudomonas aeruginosa* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Pseudomonas aeruginosa*. Consult appropriate references.^{1,5}

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. King, Ward and Raney. 1954. J. Lab. Clin. Med. 44:301.
3. Furia and Schenkel. January, 1968. Soap and Chemical Specialties.
4. Gaby and Free. 1931. J. Bacteriol. 22:349.
5. Isenberg and Garcia (ed.). 2004 (update, 2007) Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ *Pseudomonas* Isolation Agar

Cat. No. 292710 Dehydrated – 500 g

Europe

Cat. No. 257002 Prepared Plates – Pkg. of 20*

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g

228220 Bottle – 500 g

*Store at 2-8°C.