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

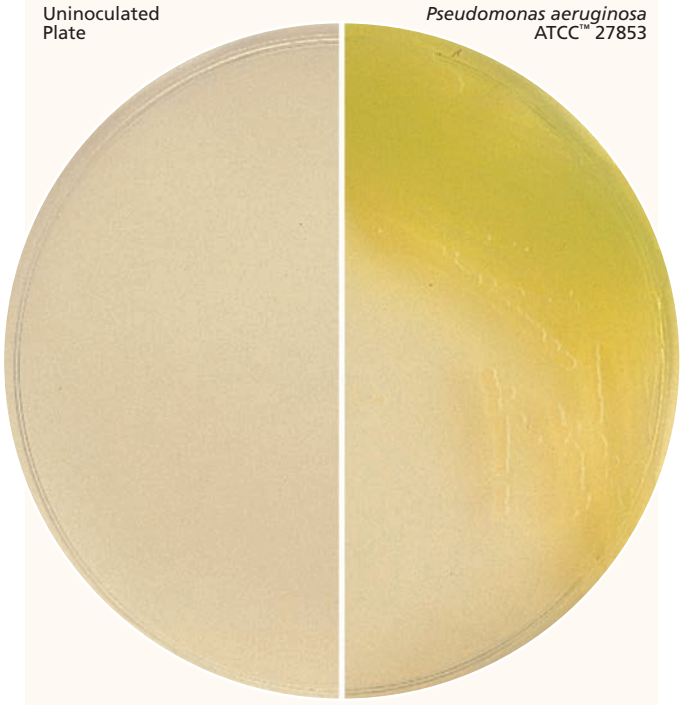
Dehydrated Appearance:	Very light beige, homogeneous, free-flowing.
Solution:	4.5% solution, soluble in purified water containing 2% glycerol upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light amber, slightly opalescent.
Reaction of 4.5% 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
Escherichia coli	25922	10^3-2×10^3	Marked to complete inhibition	–
Pseudomonas aeruginosa	10145	10^2-10^3	Good	Green to blue-green
Pseudomonas aeruginosa	27853	10^2-10^3	Good	Green to blue-green



Procedure

Inoculate the medium using the streak plate method to obtain isolated colonies. Incubate for 18-48 hours at 35 ± 2°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 Yolken (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.*