

ISP Medium 1 • ISP Medium 2 • ISP Medium 4

Intended Use

ISP Medium 1, ISP Medium 2 and ISP Medium 4 are used for characterizing *Streptomyces* species according to the International Streptomyces Project (ISP).¹

Summary and Explanation

ISP media were developed by Difco Laboratories for the International Streptomyces Project (ISP) in order to select stable properties and reproducible procedures for characterization of *Streptomyces* species.¹

ISP Medium 1 is also referred to as Tryptone-Yeast Extract Broth.

ISP Medium 2 is also referred to as Yeast Extract-Malt Extract Agar.

ISP Medium 4 is also referred to as Inorganic Salts-Starch Agar.

Principles of the Procedure

Peptone and yeast extract provide nitrogen, vitamins, carbon and amino acids in ISP Medium 1.

Yeast extract and malt extract provide nitrogen, amino acids and vitamins in ISP Medium 2. Dextrose is the carbon source. Agar is the solidifying agent.

ISP Medium 4 is composed of many inorganic salts and soluble starch to provide essential nutrients for organism growth. Agar is the solidifying agent.

Formulae

Difco™ ISP Medium 1

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	5.0 g
Yeast Extract	3.0 g

Difco™ ISP Medium 2

Approximate Formula* Per Liter	
Yeast Extract	4.0 g
Malt Extract	10.0 g
Dextrose	4.0 g
Agar	20.0 g

Difco™ ISP Medium 4

Approximate Formula* Per Liter	
Soluble Starch	10.0 g
Dipotassium Phosphate	1.0 g
Magnesium Sulfate USP	1.0 g
Sodium Chloride	1.0 g
Ammonium Sulfate	2.0 g
Calcium Carbonate	2.0 g
Ferrous Sulfate	1.0 mg
Manganous Chloride	1.0 mg
Zinc Sulfate	1.0 mg
Agar	20.0 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:
Difco™ ISP Medium 1 – 8 g;
Difco™ ISP Medium 2 – 38 g;
Difco™ ISP Medium 4 – 37 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. Mix thoroughly while dispensing ISP Medium 4.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ ISP Medium 1

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in purified water upon boiling. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.
Reaction of 0.8% Solution at 25°C:	pH 7.0 ± 0.2

Difco™ ISP Medium 2

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water 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 at 25°C:	pH 7.2 ± 0.2

Difco™ ISP Medium 4

Dehydrated Appearance:	White to light beige, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is white to off-white, opaque with precipitate.
Prepared Appearance:	White to off-white, opaque, may have a precipitate.
Reaction of 3.7% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Difco™ ISP Medium 1, ISP Medium 2 or ISP Medium 4

Prepare the medium per label directions. Inoculate tubes of prepared ISP Medium 1 with the test organisms and incubate at 30 ± 2°C for up to 96 hours. Inoculate prepared ISP Medium 2 and ISP Medium 4 with the test organisms by placing approximately 0.1 mL of inoculum near the edge of the plate. Five parallel streaks across the plate are made from this 0.1 mL of inoculum, followed by four perpendicular streaks. Incubate inoculated plates at 30 ± 2°C for 48-96 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Streptomyces albus</i>	3004	10 ² -10 ³	Good
<i>Streptomyces lavendulae</i>	8664	10 ² -10 ³	Good

Procedure

For details on the use of these media for characterization of *Streptomyces* species, consult the reference.¹ For a complete discussion on the isolation and maintenance of *Streptomyces* species refer to appropriate references.^{2,3}

Expected Results

Refer to appropriate references and procedures for results.

References

1. Shirling and Gottlieb. 1966. *Int. J. Syst. Bacteriol.* 16:313.
2. Isenberg and Garcia (ed.). 2004 (update, 2007). *Clinical microbiology procedures handbook*, 2nd ed. American Society for Microbiology, Washington, D.C.
3. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. *Manual of clinical microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ ISP Medium 1

Cat. No. 276910 Dehydrated – 500 g

Difco™ ISP Medium 2

Cat. No. 277010 Dehydrated – 500 g

Difco™ ISP Medium 4

Cat. No. 277210 Dehydrated – 500 g