



ISP Medium No. 5 (Glycerol Asparagine Agar Base)

M360

ISP Medium No. 5 (Glycerol Asparagine Agar Base) is recommended for cultivation of *Streptomyces* species as per International Streptomyces Project.

Composition**

Ingredients	Gms / Litre
L-Asparagine	1.000
Dipotassium phosphate	1.000
*Trace salt solution (ml)	1.000
Agar	20.000
1ml of Trace salt solution contains	-
Ferrous sulphate heptahydrate	0.001
Manganese chloride tetrahydrate	0.001
Zinc sulphate heptahydrate	0.001
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22.002 grams in 1000 ml distilled water containing 10 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

ISP Medium No. 5 (Glycerol Asparagine Agar Base) is based on the formulation described by Shirling and Gottlieb (1) and is used for cultivation and characterization of *Streptomyces* species as recommended by the International Streptomyces Project. Being primarily soil inhabitants, *Streptomyces* are most commonly limited to causing actinomycotic mycetoma. Areas more prone to formation of mycetomas are those that are frequently traumatized or that come into contact with soil (2).

This medium provides consistent and reproducible characteristic features of *Streptomyces*. Glycerol serves as the carbon source while asparagine is the amino acid source for the growth of *Streptomyces* species. Trace mineral requirement of *Streptomyces* is satisfied by the trace salt solution, which contains various salts. Dipotassium phosphate buffers the medium.

Quality Control

Appearance

Off-white to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 2.2% w/v aqueous solution containing 1.0% glycerol at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

M360: Cultural characteristics observed after an incubation at 25-30°C upto 15 days.

Organism

Growth

Cultural Response

<i>Streptomyces albus subsp albus</i> ATCC 3006	good-luxuriant
<i>Streptomyces lavendulae</i> ATCC 8664	good-luxuriant

Streptomyces peucetius good-luxuriant
ATCC 29050

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1. Shirling E. B. and Gottlieb D., 1966, International J. Systemic Bacteriol., 16:3.
2. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

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