ISP Medium No. 5 (Glycerol Asparagine Agar Base) M360

Intended Use:

Recommended for cultivation of *Streptomyces* species as per International *Streptomyces* Project.

Composition**

Ingredients	Gms / Litre
L-Asparagine	1.000
Dipotassium hydrogen 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 purified/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 (4) 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.

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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

Organism	Growth
Streptomyces albus subsp *albus* ATCC 3006	good-luxuriant
Streptomyces lavendulae ATCC 8664	good-luxuriant
Streptomyces peucetius ATCC 29050	good-luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
4. Shirling E. B. and Gottlieb D., 1966, International J. Systemic Bacteriol., 16:3.

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