Antibiotic Assay Medium No.12 (Nystatin Assay Agar) M280

Intended Use

Recommended for microbiological assay of Amphotericin B and Nystatin using *Saccharomyces cerevisiae* ATCC 2601.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Sodium chloride	10.000
Dextrose (Glucose)	10.000
HM peptone B #	2.500
Yeast extract	5.000
Agar	25.000
Final pH (at 25°C)	6.1±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 62.5 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

This medium is prepared from the Groove and Randall formula (1)Antifungal antibiotics like Amphotericin B and Nystatin can be assayed using this medium.

Ingredients like peptone, yeast and HM peptone B supplements essential nutrients, minerals and growth factors for the growth of test organism. Dextrose in the medium provides enhanced source of carbon and energy. Osmotic equilibrium in the medium is by sodium chloride which maintain the cell intergrity and viability. Freshly prepared plates should be used for antibiotic assays.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

Test organisms are inoculated in sterilised agar pre-cooled to 40-45°C and spread evenly over the surface of solidified base agar. Prediffusion of antibiotics for 10-20 mins in the agar by incubating at temperature below the optimal growth temperature for microorganism would facilitate better diffusion of antibiotics followed by incubation of plates for microbial growth.

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. Freshly prepared plates must be used or it may give erroneous results.

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
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.5% Agar gel.

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 6.25% w/v aqueous solution at 25°C. pH : 6.1±0.2

pH
5.90-6.30

Cultural Response
Cultural characteristics observed after an incubation at 25-30°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Saccharomyces cerevisiae ATCC 2601	50-100	luxuriant	>=70%	Amphotericin B, Nystatin

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order 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 (2,3).

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.

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