Antibiotic Assay Medium No.11

MU004

Intended Use:

Recommended for microbiological assay of antibiotics in accordance with USP.

Composition**

Ingredients	g / L
Peptone	6.000
Tryptone	4.000
Yeast extract	3.000
HM peptone B #	1.500
Dextrose (Glucose)	1.000
Agar	15.000
pH after sterilization	8.3±0.1

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

Directions

Suspend 30.5 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 or dispense as desired.

Advice: Recommended for the Microbiological assay of *Erythromycin, Netilmicin, Gentamicin, Sisomicin, Neomycin, Paromomycin.*

Principle And Interpretation

This medium is formulated in accordance to USP and CFR; and is employed to analyze the neomycin content as per FDA and the USP (1,2). It is indentical numerically with the name assigned by Grove and Randall (3). This medium provides a pH range of 8.3 while Antibiotic assay medium no.1 provides pH range of 6.5-6.7.

Peptone, tryptone, yeast and HM peptone B supply essential nutrients, vitamins, mineral, trace elements and growth factors. Dextrose in the medium serves as the carbon source for stimulating the growth of the test microorganism. Agar provides excellent medium for antibiotic diffusion and gives well defined zones of inhibition. Higher pH provides the optimal conditions for activity of antibiotic and also supports the growth of test organisms.

Type of specimen

Antibiotics as per USP

Specimen Collection and Handling

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar pre-cooled to 40-45°C and spread evenly over the surface of solidified base agar. All conditions in the microbiological assay must be controlled carefully. 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. Freshly prepared plates must be used or it may result in 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 1.5% Agar gel

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

Reaction

Reaction of 3.05% w/v aqueous solution after sterilization.

pH
8.20-8.40

Growth Promotion Test
As per US Pharmacopoeia

Cultural Response

Cultural characteristics observed after an incubation at 32-35°C for 24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
$ *Kokuria rhizophila* ATCC 9341	50-100	luxuriant	>=70%	Erythromycin
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=70%	Gentamicin,Netilmicin, Neomycin, Sisomicin, Paromomycin

Key : *- Corresponding WDCM numbers $ Formerly known as *Micrococcus luteus*

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 (4,5).

Reference

1. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436,Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242-259, (April1).
2. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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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