Antibiotic Assay Medium No. 3 (Assay Broth)

M042

Intended Use:

Recommended for microbiological assay of antibiotics.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
HM peptone B #	1.500
Yeast extract	1.500
Dextrose (Glucose)	1.000
Sodium chloride	3.500
Dipotassium hydrogen phosphate	3.680
Potassium dihydrogen phosphate	1.320
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 17.5 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C.

Advice: Recommended for the microbiological assay of Amikacin, Capreomycin, Chlortetracycline,Chloramphenicol, Cycloserine,Demeclocycline,Dihydrostreptomycin, Doxycycline, Gentamicin, Gramicidin, Kanamycin, Methacycline, Neomycin, Novobiocin, Oxytetracycline, Rolitetracycline, Streptomycin, Tetracycline, Tobramycin, Trolendomycin and Tylosin according to official methods.

Principle And Interpretation

Antibiotic Assay Medium is used in the performance of antibiotic assays. Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (1). Antibiotic Assay Medium No. 3 (Assay Broth) is used in the microbiological assay of different antibiotics in pharmaceutical and food products by the turbidimetric method. Ripperre et al reported that turbidimetric methods for determining the potency of antibiotics are inherently more accurate and more precise than agar diffusion procedures (4).

Turbidimetric antibiotic assay is based on the change or inhibition of growth of a test microorganims in a liquid medium containing a uniform concentration of an antibiotic. After incubation of the test organism in the working dilutions of the antibiotics, the amount of growth is determined by measuring the light transmittance using spectrophotometer. The concentration of antibiotic is determined by comparing amounts of growth obtained with that given by the reference standard solutions. Use of this method is appropriate only when test samples are clear.

Peptone, HM peptone B and yeast extract provides essential nutrients and growth factors for enhanced microbial growth. Sodium chloride maintains the osmotic equilibrium of the medium and retains the cell viability and cell integrity. Phosphates in the medium provide good buffering action. Dextrose serves as the carbon and energy source. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important steps for the good results.

Type of specimen

Pharmaceutical preparations

Specimen Collection and Handling

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (1).
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

Colour and Clarity of prepared medium
Light yellow coloured clear solution

Reaction
Reaction of 1.75% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 32-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Serial dilution with
Escherichia coli ATCC 10536	50-100	luxuriant	Chloramphenicol
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	Capreomycin, Dihydrostreptomycin, Streptomycin, Troleandomycin
Staphylococcus aureus ATCC 29737	50-100	luxuriant	Amikacin, Chlortetracycline, Cycloserine, Demeclocycline, Doxycycline, Kanamycin,Kanamycin sulphate, Methacycline, Oxytetracycline, Rolitetracycline, Tetracycline, Tobramycin, Tylosin, Gentamicin, Gramicidin, Neomycin, Novobiocin
Enterococcus hirae ATCC 10541	50-100	luxuriant	
Staphylococcus aureus ATCC 9144	50-100	luxuriant	Tylosin

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. Use before expiry date on the label.
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.

4. Rippere R. A.. Some principles of microbiological turbidimetric assays of antibiotics. J. Assoc. off. Anal. Chem. 1979 62(4):951-6.

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