Antibiotic Assay Medium No. 3 (Assay Broth)

M042

Antibiotic Assay Medium No. 3 (Assay Broth) is used for microbiological assay of antibiotics.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue (Peptone)	5.000
Beef extract	1.500
Yeast extract	1.500
Dextrose	1.000
Sodium chloride	3.500
Dipotassium phosphate	3.680
Potassium dihydrogen phosphate	1.320
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

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

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 (2).

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.

Peptic digest of animal tissue, beef extract 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.

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
M042: Cultural characteristics observed after an incubation at 32-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Serial dilution with
Cultural Response			
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
Enterococcus hirae ATCC 10541	50-100	luxuriant	Gentamicin,Gramicidin,Neomycin, Novobiocin
Staphylococcus aureus ATCC 9144	50-100	luxuriant	Tylosin

Storage and Shelf Life
Store below 30°C in a tightly closed container and use freshly prepared medium . Use before expiry date on label.

Reference
1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
2. 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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