Antibiotic Assay Medium No.3

MU042

Antibiotic Assay Medium No.3 is used as the broth medium in turbidimetric or serial dilution assay of a wide variety of antibiotics in accordance with United States Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Yeast extract	1.500
Beef extract	1.500
Dextrose	1.000
Sodium chloride	3.500
Dibasic potassium phosphate	3.680
Monobasic potassium phosphate	1.320
pH after sterilization	7.0±0.05

**Formula adjusted, standardized to suit performance parameters

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.

Advice : Recommended for the Microbiological assay of Amikacin, Capreomycin, Chloramphenicol, Chlortetracycline, Cycloserine, Demeclocycline, Dihydrostreptomycin, Doxycycline, Gramicidin, Kanamycin, Methacycline, Oxytetracycline Rolitetracycline, Streptomycin, Tetracycline, Tobramycin and Troleandomycin according to official methods.

Principle And Interpretation

Grove and Randall have elucidated the antibiotic assays and medias in their comprehensive treatise on antibiotic assays (1). Antibiotic assay Medium No. 3 is used as the broth medium in turbidimetric or serial dilution assay of a wide variety of antibiotics. This medium is formulated in accordance with The United States Pharmacopoeia (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.

Peptone, beef extract and yeast extract provide essential nutrients and growth factors for enhanced microbial growth. Sodium chloride maintains the osmotic equilibrium and retains the cell viability and cell intergrity. Phosphates in the medium provide good buffering action. Dextrose serves as the carbon and energy source for luxuriant growth.

Quality Control

Appearance
Cream to yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate.

Reaction
Reaction of 1.75% w/v aqueous solution at 25°C (after sterilization). pH : 7.0±0.05

pH
6.95-7.05

Growth Promotion Test
In Accordance with the harmonized method of USP.

Cultural Response
Cultural characteristics observed after incubation at specified temperature.

Cultural Response

Organism	Inoculum (CFU)	Growth	Serial dilution with	Incubation temperature / period
Cultural Response				
Escherichia coli ATCC 10536	50-100	luxuriant	Chloramphenicol	32-35°C / 24 hours
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	Capreomycin, Dihydrostreptomycin, Streptomycin, Troleandomycin	36-37.5°C / 16-24 hours
Staphylococcus aureus ATCC 29737	50-100	luxuriant	Amikacin, Chlortetracycline, Cycloserine, Demeclocycline, Doxycycline, Kanamycin,,, Lincomycin, Methacycline, Oxytetracycline, Rolitetracycline , Tetracyclin, Tobramycin,	32-35°C/ 24 hours
Enterococcus hirae ATCC 10541	50-100	luxuriant	Gramicidin	36-37.5°C / 16-18 hours
Staphylococcus aureus ATCC 9144	50-100	luxuriant	Tylosin	35-39°C/16-18 hours

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

Reference
1. Grove and Randall, 1955, Assay Methods of Antibiotics, Medical Encyclopedia, Inc. New York
2. United States Pharmacopoeia 2011, US Pharmacopoeial Convention, Inc., Rockville, MD.

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