



## Antibiotic Assay Medium No.1

MU003

Antibiotic Assay Medium No.1 is used for microbiological assay of  $\beta$ -lactam and other antibiotics in accordance with United States Pharmacopoeia.

### Composition\*\*

Ingredients	Gms / Litre
Peptone	6.000
Pancreatic digest of casein	4.000
Yeast extract	3.000
Beef extract	1.500
Dextrose	1.000
Agar	15.000
pH after sterilization	6.6 $\pm$ 0.1

\*\*Formula adjusted, standardized to suit performance parameters

### 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.

*Advice : Recommended for the microbiological assay of Bacitracin, Nafcillin, Cephalothin, Cephaperin, Cloxacillin, Novobiocin, Penicillin-G ..*

### Principle And Interpretation

This medium is also used as inoculum and maintenance medium for different test organisms for antibiotic assays. Composition of this medium is in accordance with US Pharmacopoeia (1) and is recommended by FDA(2) and identified numerically with the name assigned by Grove and Randall (3).

Essential nutrients, vitamins, mineral, trace elements and growth factors are supplied by peptone, pancreatic digest of casein, yeast and beef extract. 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.

Freshly prepared plates should be preferably used for assaying antibiotics. Test organisms is 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. One of the critical and important step for obtaining good results is use of appropriate standard culture media.

### Quality Control

#### Appearance

Cream to yellow coloured homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Yellow coloured slightly opalescent gel forms in Petri plates.

#### pH

6.50-6.70

#### Cultural Response

MU003: Cultural characteristics observed after an incubation at specified temperature and period.

Organism	Inoculum (CFU)	Growth	Recovery	Inoculum medium	Incubation temperature / period	Antibiotics assayed
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	good-luxuriant	>=50%	Colistimethate sodium, Colistin, Polymyxin B	32-35°C/ 24 hours	
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	>=70%	Chloramphenicol	32-35°C/ 24 hours	
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	good-luxuriant	>=50%	Capreomycin, Dihydrostreptomycin, Neomycin, Streptomycin, Troleandomycin	36-37.5°C/ 16-24 hours	
<i>Micrococcus luteus</i> ATCC 9341	50-100	luxuriant	>=70%	Erythromycin	32-35°C/ 24 hours	
<i>Micrococcus luteus</i> ATCC 10240	50-100	good-luxuriant	>=70%	Bacitracin	32-35°C/ 24 hours	Bacitracin
<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	luxuriant	>=70%	Carbenicillin	36-37.5°C/ 16-24 hours	
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	good-luxuriant	>=70%	Gentamicin, Netilmicin, Neomycin, Novobiocin, Paromomycin, Sisomicin	32-35°C/ 24 hours	Novobiocin
<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant	>=70%	Amikacin, Cephalothin, Cephaperin, Chlortetracycline, Cloxacillin, Cycloserine, Demeclocycline, Kanamycin, Methacycline, Nafcillin, Penicillin-G, Rolitetracycline, Tetracycline, Tobramycin, Tylosin	32-35°C/ 24 hours	Cephalothin, Cephaperin, Cloxacillin, Nafcillin, Penicillin-G,

## Storage and Shelf Life

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

## Reference

1. United States Pharmacopoeia USP 2011, US Pharmacopoeial Convention, Inc., Rockville, MD.
2. 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, (April 1).
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopaedia, Inc. New York

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