

## Antibiotic Assay Medium No.1 (Seed Agar), Granulated

**GM003**

Antibiotic Assay Medium No.1 (Seed Agar), granulated is used in the microbiological assay of beta-lactam and other antibiotics.

### Composition\*\*

Ingredients	Gms / Litre
Peptic digest of animal tissue (Peptone)	6.000
Casein enzymic hydrolysate	4.000
Yeast extract	3.000
Beef extract	1.500
Dextrose	1.000
Agar	15.000
Final pH ( at 25°C)	6.6±0.2

\*\*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. Cool to 45-50°C. Mix well and pour into sterile Petri plates or dispense as desired.

*Advice : Recommended as a inoculum medium for Amikacin, Bacitracin, Capreomycin, Cephalothin, Cephaperin, Chloramphenicol, Chlortetracycline, Cloxacillin Cycloserine, Colistimethate sodium, Colistin, Demeclocycline, Dicloxacillin, Dihydrostreptomycin, Erythromycin, Framycetin, Gentamicin, Kanamycin, Kanamycin B, Kanamycin sulphate, Lymecycline, Methacycline, Methicillin, Nafcillin, Neomycin, Netilmicin, Novobiocin, Oxacillin, Oxytetracycline, Paromomycin, Penicillin-G, Phenoxy-methyl Penicillin, Rifamycin sodium Rolitetracycline, Sisomycin Spiramycin, Streptomycin Tetracycline, Tobramycin, Troleandomycin, Tylosin*

### Principle And Interpretation

The potency of an antibiotic can be determined by chemical, physical and biological means. An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganisms. Biological tests offer the most convenient means of performing an assay (1), since a reduction in the antimicrobial activity of a specific antibiotic reveals changes not usually displayed by chemical methods (2). Antibacterial susceptibility testing may be performed by either dilution (turbidimetric) or diffusion methods. The choice of methodology is often based on many factors, including relative ease of performance, flexibility and use of automated or semi-automated devices for both identification and susceptibility testing (3). Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (4). Antibiotic Assay Medium No.1 is used in the microbiological assay of β-lactam and other antibiotics. These media are prepared according to the specifications detailed in various pharmacopoeias (2,5,6) and by the FDA (7).

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar cooled to 40-45°C and spread evenly over the surface of solidified base agar. After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic. All conditions in the microbiological assay must be carefully controlled. The use of standard culture media in the test is one of the important steps for good results.

Nutrients and growth factors are supplied by the ingredients like peptic digest of animal tissue, casein enzymic hydrolysate, yeast extract and beef extract. Dextrose is supplemented as a carbon and energy source.

### Quality Control

#### Appearance

Cream to yellow coloured granular medium

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent gel forms in Petri plates.

### Reaction

Reaction of 3.05% w/v aqueous solution at 25°C. pH : 6.6±0.2

### pH

6.40-6.80

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

### Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Inoculum medium	Assay medium	Inoculum & Assay medium
<i>Bacillus subtilis</i> ATCC 6633	50-100	luxuriant	≥70%	Framycetin, Josamycin, Josamycin propionate, Kanamycin B, Spiramycin, Streptomycin, Vancomycin	Streptomycin, Vancomycin	
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	luxuriant	≥50%	Colistimethate sodium, Colistin, Polymyxin B		
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	≥70%	Chloramphenicol		
<i>Bacillus cereus</i> var <i>mycoides</i> ATCC 11778	50-100	luxuriant	≥70%	Oxytetracycline, Tetracycline		
<i>Bacillus pumilis</i> ATCC 14884	50-100	luxuriant	≥70%	Chlortetracycline, Framycetin, Kanamycin sulphate		
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	≥70%	Capreomycin, Dihydrostreptomycin, Neomycin, Streptomycin, Troleandomycin		
<i>Micrococcus luteus</i> ATCC 9341	50-100	luxuriant	≥70%	Erythromycin, Rifamycin sodium	Erythromycin	
<i>Micrococcus luteus</i> ATCC 10240	50-100	luxuriant	≥70%			Bacitracin
<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	luxuriant	≥70%	Carbenicillin		
<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant	≥70%	Amikacin, Cephothin, Cephapirin, Chlotetracycline, Cloxacillin, Cycloserine, Demeclocycline, Doxycycline, Kanamycin, Methacycline, Nafcillin, Oxytetracycline, Penicillin-G, Rolitetracycline, Tetracycline, Tobramycin, Tylosin	Cephalothin, Cephapirin, Cloxacillin, Nafcillin, Penicillin-G	
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	luxuriant	≥70%	Gentamicin, Neomycin, Netilmicin, Novobiocin, Sisomycin, Paromomycin		

### 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. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Edi, Tata McGraw-Hill Publishing Company Ltd, New Delhi
2. The United States Pharmacopoeia, 2014, The United States Pharmacopoeial Convention, Rockville, MD.
3. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Eds.), 2007, Manual of Clinical Microbiology, 9th Ed., American Society for Microbiology, Washington, D.C.
4. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
5. European Pharmacopoeia, 2014, European Department for the Quality of Medicines of Council of Europe.
6. British Pharmacopoeia, 2014, The Stationery Office British Pharmacopoeia.
7. 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).

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