

**Antibiotic Assay Medium No. 2 (Base Agar), Granulated****GM005**

Antibiotic Medium No. 2 (Base Agar) , granulated is used as a basal medium for microbiological assay of antibiotics.

**Composition\*\***

<b>Ingredients</b>	<b>Gms / Litre</b>
Peptic digest of animal tissue (Peptone)	6.000
Beef extract	1.500
Yeast extract	3.000
Agar	15.000
Final pH ( at 25°C)	6.6±0.2

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

**Directions**

Suspend 25.5 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 for the microbiological assay of Amphomycin, Bacitracin, Cephalexin, Cephaloglycin, Cephaloridine, Cephalothin, Cloxacillin, Dicloxacillin, Methicillin, Nafcillin, Novobiocin, Oxacillin, Penicillin-G, Phenoxymethyl Penicillin, Spiramycin and Rifampicin

**Principle And Interpretation**

This medium is commonly used as base agar for microbiological agar diffusion assays for wide variety of antibiotics. Agar diffusion assays can be performed by cylinders, punched-hole or paper disc tests. This medium is identical numerically with the name assigned by Grove and Randall (1) This medium is equivalent to Antibiotic Assay Medium No.B as per Indian Pharmacopoeia (3). Peptic digest of animal tissue, yeast and beef extract provide the nitrogenous, vitamins and mineral requirement for the growth of test organisms. This medium provides solidified substratum for growth of organisms and supports the overlaying of soft agar.

To perform the antibiotic assay the Antibiotic assay medium No.2 is used as Base Agar. This medium should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, Antibiotic assay medium No.1 as seed agar, inoculated with the standardized culture can be overlaid. Even distribution of the layer is important.

**Quality Control****Appearance**

Cream to yellow coloured granular medium

**Gelling**

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**

Amber coloured, clear to slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 2.55% 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 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed	Basal layer
<b>Cultural Response</b>					
<i>Bacillus subtilis</i> ATCC 6633	50-100	luxuriant	>=70%	Spiramycin	
<i>Micrococcus luteus</i> ATCC 10240	50-100	luxuriant	>=70%		Bacitracin
<i>Staphylococcus aureus</i> ATCC 9144	50-100	luxuriant	>=70%		Tylosin
<i>Staphylococcus aureus</i> ATCC 29737	50-100	luxuriant	>=70%		Amikacin,Cephalothin,Cephapirin, Chlortetracycline, Nafcillin,Oxytetracycline, Rolitetracycline, Tetracycline
					Cloxacillin,Cycloserine,Demeclocycline, Doxycycline,Kanamycin,Methacycline,
<i>Staphylococcus epidermidis</i> ATCC 12228	50-100	good-luxuriant	>=70%		
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	luxuriant	>=70%		Capreomycin, Streptomycin, Troleandomycin
<i>Enterococcus hirae</i> ATCC 10541	50-100	luxuriant	>=70%		Gramicidin,Thiostrepton,Tobramycin
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	>=70%		Chloramphenicol, Spectinomycin

### 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.1.
2. Indian Pharmacopoeia 2007, Ministry of Health and Family Welfare, Govt. of India, Delhi.

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