

## Technical Information

### Antibiotic Assay Medium No. 2 (Base Agar)

**Product Code : DM1005**

**Application:** - Antibiotic Medium No. 2 (Base Agar) is used as a basal medium for microbiological assay of antibiotics.

#### Composition\*\*

Ingredients	Gms / Litre
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

#### Principle & Interpretation

This medium is commonly used as base agar for microbiological agar diffusion assays for larger number of antibiotics. Agar diffusion assays can be performed by cylinders, punched-hole or paper disc tests. This medium is numerically identical with the name assigned by Grove and Randall <sup>(1)</sup> and is equivalent to Antibiotic Assay Medium No.B as per Indian Pharmacopoeia <sup>(3)</sup>. 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 overlay 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.

#### Methodology

Suspend 25.5 grams of powder media in 1000 ml distilled water. Shake well and heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Advice: Recommended for the microbiological assay of Spiramycin.

#### Quality Control

##### Physical Appearance

Cream to yellow homogeneous free flowing powder

##### 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 range 6.40-6.80

#### Cultural Response/ characteristics

DM 1005: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed	Basal layer
<i>Bacillus subtilis</i> ATCC 6633	50-100	luxuriant	>=70%	Spiramycin	
<i>Micrococcus luteus</i> ATCC 10240					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, Chlorotetra Nafcillin, Oxytetracycline, Rolitetracycline, Tetracycline
<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

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

**Prepared Media:** 2-8° in sealable plastic bags for 2-5 days.

## Further Reading

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.

## Disclaimer :

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