

TM 359 – ANTIBIOTIC ASSAY MEDIUM NO.2 (BASE AGAR)

INTENDED USE

For microbiological assay of antibiotics.

PRODUCT SUMMARY AND EXPLANATION

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 this medium is equivalent to Antibiotic Assay Medium No.B as per Indian Pharmacopoeia.

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.

COMPOSITION

Ingredients	Gms / Ltr		
Peptone	6.000		
Beef extract	1.500		
Yeast extract	3.000		
Agar	15.000		

PRINCIPLE

Peptone, yeast and beef extract provide the nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and mineral requirement for the growth of test organisms. This medium provides solidified substratum for growth of organisms and supports the over layering of soft agar.

INSTRUCTION FOR USE

- Dissolve 25.5 grams in 1000 ml purified/ distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C.
- Mix well and pour into sterile Petri plates.

Advice: Recommended for the microbiological assay of Spiramycin.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance of prepared medium : Amber coloured, clear to slightly opalescent gel forms in Petri plates.

pH (at 25°C) : 6.6±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC Inoculum (CFU/ml)	croorganism /	Growth	Recovery	Antibiotics assayed	Basal layer	Incubati on Tempera ture	Incubatio n Period
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Bacillus subtilis subsp. spizizenii	6633	50-100	Luxuriant	>=70%	Spiramycin	-	35-37°C	18-48 Hours
Micrococcus Iuteus	10240	50-100	Luxuriant	>=70%	-	Bacitracin	35-37°C	18-48 Hours
Staphylococcus aureus	9144	50-100	Luxuriant	>=70%	-	Tylosin	35-37°C	18-48 Hours
Staphylococcus aureus	29737	50-100	Luxuriant	>=70%	-	Amikacin,Cephalo thin,Cephapir in, Chlortetracycline, Nafcillin, Oxytetracycline,R olitetracycline	35-37°C	18-48 Hours
Staphylococcus epidermidis	12228	50-100	Good- luxuriant	>=50%	-	Tetracycline	35-37°C	18-48 Hours
Klebsiella pneumoniae	10031	50-100	Luxuriant	>=70%	-	Capreomycin, Streptomycin, Troleandomycin, Gramicidin, Thiostrepton,Tob ramycin	35-37°C	18-48 Hours
Enterococcus hirae	10541	50-100	Luxuriant	>=70%	-	-	35-37°C	18-48 Hours
Escherichia coli	10536	50-100	Luxuriant	>=70%	-	Chloramphenicol, Spectinomycin	35-37°C	18-48 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- 1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.1.
- 2. Indian Pharmacopoeia 2018, Ministry of Health and Family Welfare, Govt. of India, Delhi.
- **3.** Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
- 4. Jorgensen, J.H., Pfaller, M.A., Carrol, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.







































NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. *For Lab Use Only

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