

ANTIBIOTIC ASSAY MEDIUM NO. 2 (Base Agar)**TM 359**

For microbiological assay of antibiotics

Composition

Ingredients	Gms/Ltr.
Agar	15.00
Pancreatic digest of animal tissue	6.00
Yeast extract	3.00
Beef extract	1.50

* Dehydrated powder, hygroscopic in nature, store, in a dry place in tightly- sealed containers below 25°C and protect from direct Sunlight.

Instructions for Use

Dissolve 25.50gms 1000ml distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Boil for one minute. Distribute into appropriate containers. Sterilize by autoclaving at 15psi (121°C) for 15 minutes.

Appearance: Light amber colour, clear to slightly opalescent gel

pH (at 25°C): 6.6 ± 0.2

Principle

ANTIBIOTIC ASSAY MEDIUM No. 2 (BASE AGAR) is used for testing the potency of antibiotics by the inhibitory effects on microorganisms. The cylinder assay plate is based on the diffusions of the antibiotics under test, measuring the size of the zones of inhibition of the microorganisms. This media is formulated as according to the guidelines of the USP. Medium contains Pancreatic digest of animal tissue, Beef extract and Yeast extract those provides nitrogen, carbon and vitamins (particularly B group) sources to the medium. Dextrose is added as a source of carbohydrate. Agar is a solidifying agent.

1. ASSAY PLATE DIFFUSION METHOD: Cylinders, or Paper disc tests

ANTIBIOTIC ASSAY MEDIUM No. 2 (Base Agar) is used as an inoculum substrate (20 X 100 mm). Cover the lid of the petri plate. Once the medium has solidified, use 4ml of the seed layer to inoculate with the standard culture to be used for the particular antibiotic to be tested. Once the seed layer is solid, the cylinders are placed evenly spaced on a 2.5 - 3 mm radius, for the antibiotic solutions under test. The standard sample is added as required.

This petri plate is incubated for 24 hours at 35 - 2° C. Later, the zones of inhibition are observed, measured and compared with the calibration curve determined by adding known amounts of the same antibiotic used in the same plate.

Interpretation

Cultural characteristics observed after inoculating (10³CFU/ml), on incubation at 35 ± 37°C for 18 – 24 hours.

PRODUCT DATA SHEET

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth	Inhibition zones
<i>Staphylococcus aureus</i>	6538	10 ³	Good	Methicillin, Penicillin, Oxacillin, Nafcillin
<i>Staphylococcus epidermidis</i>	12228	10 ³	Good	Novobiocin
<i>Micrococcus luteus</i>	10240	10 ³	Good	Bacitracin

References

1. Grove and Randall. Assay Methods of Antibiotics, Medical Encyclopedia Inc. New York. United States Pharmacopoeia Convention.
2. The United States Pharmacopoeia, 23rd Ed. Biological Tests and Assays, p. 1690-1696.
3. The United States Pharmacopoeia Convention, Rockville, Md. XXVI, Chapter "Biological Tests and Assays". (1955).
4. United States pharmacopoeia Forster, J.W., a Woodruff, H.B.: Microbial aspects of penicillin - J.Bact., 46: 187 - 202. (1943).