ANTIBIOTIC AGAR n°11

Medium for determining antibiotic potency by the microbiological assay technique.

TYPICAL FORMULA (g/L)

Beef Extract	1.5
Yeast Extract	3.0
Casein Peptone	4.0
Peptone	6.0
Dextrose	1.0
Agar	15.0
Final pH 7.9 ± 0.1	

DESCRIPTION

ANTIBIOTIC AGAR n°11 is used for determining antibiotic potency by the microbiological assay technique and is conform with specifications of The United States Pharmacopeia (USP).

PRINCIPLE

Cylinder Plate Assay

This method is used in the assay of commercial preparations of antibiotics and in the quantitative determination of antibiotics in body fluids, animal feeds and other materials. It is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. The diameter of a zone of inhibition after incubation depends, in part, on the concentration or activity of the antibiotic.

Turbidimetric Assay

This method is based on the inhibition of growth of a microbial culture in a fluid medium containing a uniform solution of an antibiotic. Turbidimetric determinations have the advantage of requiring a short incubation period, providing test results after 3 or 4 hours. However, the presence of solvents or other inhibitory materials may influence turbidimetric assays more markedly than cylinder plate assays. Use of this method is appropriate only when test samples are clear.

PREPARATION

Suspend 30.5 g of powder in 1 litre of distilled or deionized water. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Raise the pH of Antibiotic Medium 11 to 8.3 ± 0.1, cooling the base to 45-50°C and adding NaOH. Dispense into final containers. Test samples of the finished product for performance using stable, typical control cultures.

TECHNIQUE

Prepare the inoculum for assay by washing growth from a fresh 24-48 hour agar slant using sterile purified water and further dilute the culture to obtain the desired organism concentration.

Cylinder Plate Assay

Use 20 × 100 mm glass or plastic Petri dishes with sufficient depth so that cylinders used in the assay will not be pushed into the medium by the cover. Use stainless steel or porcelain assay cylinders having the following dimensions (± 0.1 mm): 8 mm outside diameter, 6 mm inside diameter and 10 mm long.

To assure accurate assays, work on a level surface to obtain uniformly thick base and seed layers in the Petri dish. Allow the base layer to solidify and then overlay the seed layer containing a proper concentration of the test organism. The amount of medium in the layers varies for different antibiotics, with most assays specifying a 21 mL base layer and a 4 mL seed layer. In any case, dishes with flat bottoms are required to assure complete coverage of the bottom of the dish when small amounts of base medium are used. Tilt the plate to obtain even coverage of the base layer by the seed layer and allow it to solidify in a level position. Plates should be used the same day as prepared.

Turbidimetric Assay

Use glass or plastic test tubes (i.e., 16 × 125 mm or 18 × 150 mm) that are relatively uniform in length, diameter and thickness. Prepare working dilutions of the antibiotic reference standards in specific concentrations. To a 1 mL quantity of each solution in a suitable tube, add 9 mL of inoculated broth, as required. Prepare similar solutions of the assay materials containing approximately the same amounts of antibiotic activity and place in tubes. Incubate the tubes for 3-4 hours at the required temperature, generally in a water bath. At the end of the incubation period, stop growth by adding 0.5 mL of 1:3 formalin. Determine the amount of growth by measuring light transmittance with a suitable spectrophotometer. Determine the concentration of the antibiotic by comparing the growth obtained with that given by reference standard solutions.

INTERPRETATION OF RESULTS

Refer to appropriate procedures for results.

STORAGE

10-25°C away from light, until the expiry date on the label or until signs of deterioration or contamination are evident.

WARNING and PRECAUTIONS

The product is not classified as hazardous by current legislation and does not contain harmful substances in concentrations of ≥1%. The product is designed for *In vitro* diagnostic use and must be used only by properly trained operators.

DISPOSAL of WASTE

Disposal of waste must be carried out according to national and local regulations in force.

REFERENCES

1. *Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.*
2. *United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.*
3. *Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. 1. AOAC International, Gaithersburg, Md.*
4. *Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett ii:177.*

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PRODUCT SPECIFICATIONS

NAME
ANTIBIOTIC AGAR n°11

PRESENTATION
610315: bottle containing 500 g of dehydrated powder.
620315: bottle containing 100 g of dehydrated powder.

STORAGE
10-25°C

PACKAGING

Code	Content	Packaging
610315	500 g	• 500 g of powder in plastic bottle
620315	100 g	• 100 g of powder in plastic bottle

pH OF THE MEDIUM
7.9 ± 0.1

USE
ANTIBIOTIC AGAR n°11 is used for determining antibiotic potency by the microbiological assay technique and is conform with specifications of The United States Pharmacopeia (USP).

TECHNIQUE
Refer to technical sheet of the product.

APPEARANCE of the MEDIUM
Dehydrated medium
Appearance: free-flowing, homogeneous.
Colour: beige.
Prepared medium
Appearance: slightly opalescent.
Colour: light to medium amber.

SHELFLIFE
4 year

QUALITY CONTROL
1. Control of general characteristics, label and print
2. Sterility control
   7 days at 25 ± 1°C, in aerobiosis
   7 days at 36 ± 1°C, in aerobiosis
3. Microbiological control
   Inoculum for productivity: 30-300 UFC/ml
   Inoculation by the pour plate method and incubation for 40-48 h at 30 ± 2 °C.

Microorganism		Growth
Micrococcus luteus	ATCC 9341	Good
Staphylococcus epidermidis	ATCC 12228	Good

TABLE OF SYMBOLS

LOT Batch code	Temperature limitation	Manufacturer	Contains sufficient for <n> tests	IVD *In vitro* Diagnostic Medical Device
REF Catalogue number	Keep away from heat	Use by	Caution, consult accompanying documents	

CE IVD

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