
Product Name : Antibiotic Assay Broth No.3

Liquid medium used for the determination potency of antibiotics by the microbial assay technique following the USP specifications.

FORMULA (G/L)

Peptone.....	5.0
Yeast Extract.....	1.5
Beef Extract.....	1.5
Sodium Chloride.....	3.5
Dextrose.....	1.0
Dipotassium Phosphate.....	3.68
Monopotassium Phosphate.....	1.32
Final pH = 7.0 ± 0.2 at 25°C.	

DIRECTIONS

Suspend 17.5 G of powder in 1 L of distilled or deionized water. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Dispense into final tubes. Autoclave at 121°C for 15 minutes.

DESCRIPTION

Antibiotic Assay Broth No.3 is used for determining antibiotic potency by the microbiological assay technique. The medium is conform with specifications of The United States Pharmacopeia (USP).

TECHNIQUE

Prepare the inoculum for assay by washing growth from a fresh 24-48 hour medium 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.

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QUALITY CONTROL

Dehydrated medium

Appearance: free-flowing, homogeneous.

Color: tan.

Prepared medium

Appearance: clear to slightly hazy.

Color: light to medium amber.

Incubation conditions: $25 \pm 2^\circ \text{C}$ for the *Saccharomyces cerevisiae* and $35 \pm 2^\circ \text{C}$ for the remaining organisms for 7days.

Microorganism	ATCC	Growth
<i>Bacillus subtilis</i>	6633	good
<i>Escherichia coli</i>	10536	good
<i>Micrococcus luteus</i>	9341	good
<i>Saccharomyces cerevisiae</i>	9763	good
<i>Staphylococcus aureus</i>	6538P	good

STORAGE

The powder is very hygroscopic: store the powder at room temperature, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident. Store prepared medium at 2-6°C.

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. Foster and Woodruff. (1943). J. Bacteriol. 46:187.

PACKAGING

Cat. No : MB-A1316
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500 G