# Antibiotic Medium Nº 3

Cat. 1534

Standard medium for use in antibiotic assays.

## Practical information

| Aplications | Categories |
|---|---|
| Antibiotic Assay | General use |

Industry: Pharmaceutical/Veterinary

## Principles and uses

Antibiotic Medium Nº 3 is a standard medium prepared for use in antibiotic assays.

The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitoty effect on microorganisms. Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods. Antibiotic assays are performed by the cylinder plate method. This method, first described by Abraham et al. for the assay of penicillin, is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. The diameter of an inhibition zone after incubation depends, in part, on the concentration or activity of the antibiotic.

Antibiotic Medium Nº 3 can be used with the following microbiological methods for Antibiotic Assays:
1. Cylinder method in plates.
2. Serial dilution method.
3. Turbidimetric method.

In the cylinder method in plates, Antibiotic Medium Nº 3 is used to resuspend the inoculum in the potency assay for penicillin, erythromycin, neomycin, chlortetracycline and chloramphenicol.

Lastly, this medium can also be used in the turbidimetric determination of the potency of bacitracin, streptomycin and terramycin. The turbidimetric method is based on the inhibition of growth of a microbial culture in a fluid medium containing a uniform solution of an antibiotic. Use of this method is appropriate only when test samples are clear.

Gelatin peptone, yeast extract and beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Potassium phosphates act as a buffer system. Dextrose is the fermentable carbohydrate providing carbon and energy. Bacteriological agar is the solidifying agent.

## Formula in g/L

| | | | |
|---|---|---|---|
| Beef extract | 1,5 | Dextrose | 1 |
| Dipotassium phosphate | 3,68 | Gelatin peptone | 5 |
| Monopotassium phosphate | 1,32 | Sodium chloride | 3,5 |
| Yeast extract | 1,5 | | |

## Preparation

Suspend 17,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize at 121 ºC for 15 minutes. Distribute into appropriate containers.

## Instructions for use

Cylinder plate assay:
- Maintain stock cultures on agar slants.
- Prepare the inoculum for assay by washing a fresh 24-48 hours agar slant using the Antibiotoc Medium Nº 3 or sterile purified water and further dilute the culture to obtain the desired organism concentration.
- Inoculate the Antibiotic Medium.
- Put four or six assay cylinders on the plate and incubate at 35±2 ºC for 24-48 hours.
- Plates should be used the same day as prepared.

Turbidimetric assay:

- Wash the growth of a fresh slant agar with the Anibiotic Medium Nº 3.
- Dilute the broth as required.
- Following the reference procedures, prepare working dilutions of the antibiotic reference standard in specific concentrations.
- Incubate tubes at 35 ºC for 3-4 hours and then stop the growth with a 0,5 ml of 1:3 diluted formalin.
- Read with a suitable spectrophometer and compare the growth with the given reference standard solutions.

## Quality control

| Solubility | Appareance | Color of the dehydrated medium | Color of the prepared medium | Final pH (25ºC) |
|---|---|---|---|---|
| w/o rests | Fine powder | Beige | Clear amber | 7,0±0,2 |

## Microbiological test

Incubation conditions: (35± 2 ºC / 24-48 h).

| Microrganisms | Specification | Characteristic reaction |
|---|---|---|
| Klebsiella pneumoniae ATCC 10031 | Good growth | Inhibition zones: Streptomycin |
| Staphylococcus aureus ATCC 6538 | Good growth | Inhibition zones: Kanamycin, Neomycin |
| Micrococcus luteus ATCC 9341 | Good growth | Inhibition zones: Erythromycin |

## Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

## Bibliography

Grove and Randall. Assay Methods of Antibiotics, Medical Encyclopedia Inc. New York 1955. United States Pharmacopoeia Convention. 1955. The United States Pharmacopoeia, 23rd Ed. Biological Tests and Assays, p. 1690-1696. The United States Pharmacopoeia Convention, Rockville, Md.