

70184 Antibiotic Broth (Penassay Broth; Grove and Randall Antibiotic Broth; Antibiotic Medium No 3; Assay Broth) NutriSelect® plus

Used in antibiotic assay work (USP, FDA, Ph Eur) according to Grove & Randall; used in the turbidimetric assay of e.g. penicillin, bacitracin and kanamycin with *S. aureus* and for the cylinder assay of e.g. erythromycin and chloramphenicol with *M. luteus*.

Composition:

Ingredients	Grams/Litre
Peptone (from meat)	5.0
Yeast extract	1.5
Meat extract	1.5
Dextrose	1.0
Sodium chloride	3.5
Dipotassium hydrogen phosphate	3.68
Potassium dihydrogen phosphate	1.32

Final pH 7.0 +/- 0.2 at 25°C

Store dehydrated powder between 10-30°C in a tightly closed container and use freshly prepared media. Protect from moisture and light by keeping container in a low humidity environment. Use before expiry date on the label.

Appearance(color): Faint Yellow to yellow to faint beige to beige, free flowing powder
Color and Clarity: Light yellow coloured clear solution

Directions:

Dissolve 17.5 g in 1 litre of warm (60 °C) distilled water and sterilize by autoclaving at 121°C for 15 minutes.

Principle and Interpretation:

The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms (1). Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods (1). Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay.

Antibiotic assay media are prepared according to the specifications of the USP (1), European Pharmacopeia (2) and AOAC International (5). Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (4).

Antibiotic Assay Medium No. 3 (Assay Broth) is used in the microbiological assay of different antibiotics in pharmaceutical and food products by the turbidimetric method. *Ripperre et al* reported that turbidimetric methods for determining the potency of antibiotics are inherently more accurate and more precise than agar diffusion procedures (3).

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 (1). After incubation of the test organism in the working dilutions of the antibiotics, the amount of growth is determined by measuring the light transmittance using spectrophotometer. The concentration of antibiotic is determined by comparing amounts of growth obtained with that given by the reference standard solutions. Turbidimetric determinations requires short incubation period, providing test results after 3 or 4 hours. However, the presence of



solvents or other inhibitory materials may effect turbidimetric assays more considerably than cylinder plate assays. Use of this method is appropriate only when test samples are clear.

Peptone and yeast extract provides essential nutrients and growth factors for enhanced microbial growth. Sodium chloride maintains the osmotic balance of the medium and retains the cell viability and cell integrity. Phosphates in the medium provide good buffering action. Dextrose serves as the carbon and energy source. The use of standardized culture media and careful control of all test conditions are fundamental requisites in the microbiological assay of antibiotics in order to achieve satisfactory test results.

Cultural characteristics observed after an incubation of 18-24 hrs hours at 32-37°C.

Organisms (ATCC/WDCM)	Inoculum (CFU)	Growth	Serial dilution with
<i>Escherichia coli</i> (10536/-)	50-100	+++	Chloramphenicol
<i>Klebsiella pneumoniae</i> (10031/-)	50-100	+++	Capreomycin, Dihydrostreptomycin, Streptomycin, Troleandomycin
<i>Staphylococcus aureus</i> (29737/-)	50-100	+++	Amikacin, Chlortetracycline, Cycloserine, Demeclocycline, Doxycycline, Kanamycin, Kanamycin sulphate, Methacycline, Oxytetracycline, Rolitetracycline, Tetracycline, Tobramycin, Tylosin, Gentamicin, Gramicidin, Neomycin, Novobiocin
<i>Enterococcus hirae</i> (10541/-)	50-100	+++	
<i>Staphylococcus aureus</i> (9144/-)	50-100	+++	Tylosin

References:

1. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md
2. Council of Europe. 2002. European pharmacopeia, 4th ed. Council of Europe, Strasbourg, France.
3. Rippere R. A.. Some principles of microbiological turbidimetric assays of antibiotics. J. Assoc. off. Anal. Chem. 1979 62(4):951-6.
4. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New Yor
5. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. 1. AOAC International, Gaithersburg, Md

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

