

Antibiotic Assay Medium No.3 (DM016U)

Intended Use

Antibiotic Assay Medium No.3 (DM016U) is recommended as a broth medium for turbidometric or serial dilution assay of a wide variety of antibiotics, in compliance with USP.

Product Summary and Explanation

The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.⁽¹⁾ The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.⁽²⁾ Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.⁽²⁾ Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. 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⁾ This medium is formulated in accordance with The United States Pharmacopoeia.⁽⁴⁾

Principles of the Procedure

Antibiotic Assay Medium No. 3 contains peptic digest of animal tissue, beef extract which provides carbon, nitrogen and other essential nutrients and growth factors for enhanced microbial growth. Yeast extract is a source of B complex vitamins. Dextrose stimulates the growth by providing carbon and energy. Sodium chloride maintains the osmotic equilibrium of the medium and retains the cell viability and cell integrity. Phosphates in the medium enhance the buffering action.

Turbidimetric Assay

The turbidimetric method is based on the change or inhibition of growth of a microbial culture in a liquid 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. 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. Use of this method is appropriate only when test samples are clear.

Note:

Recommended for the Microbiological assay of Amikacin, Capreomycin, Chloramphenicol, Chlorotetracycline, Cycloserine, Demeclocycline, Dihydrostreptomycin, Doxycycline, Gramicidin, Kanamycin, Lincomycin, Meclocycline, Methacycline, Minocycline, Oxytetracycline, Rolitetracycline, Spectinomycin, Streptomycin, Tetracycline, Tobramycin, Troleandomycin, Tyrothricin and viomycin according to official methods.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue (Peptone)	5.00
Beef extract	1.50
Yeast extract	1.50
Dextrose	1.00
Sodium chloride	3.50
Dipotassium phosphate	3.68
Potassium dihydrogen phosphate	1.32
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. All conditions in the microbiological assay must be controlled carefully.
4. The use of standard culture medium in the test is one of the important steps for obtaining good results.

Directions

1. Suspend 17.5 grams of the medium in one liter of purified/ distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear solution without any precipitate
Reaction of 1.75% Solution	pH: 7.0 ± 0.2 at 25°C
Gel Strength	Not Applicable

Growth Promotion Test

In Accordance with the harmonized method of USP.

Expected Cultural Response: Cultural characteristics observed after incubation at specified temperature.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Serial dilution With	Incubation temperature / period
1.	<i>Escherichia coli</i> ATCC 10536	50 -100	good-luxuriant	Chloramphenicol	32-35°C / 24 hours
2.	<i>Klebsiella pneumoniae</i> ATCC 10031	50 -100	good-luxuriant	Capreomycin, Dihydrostreptomycin, Streptomycin, Troleandomycin	36-37.5°C / 16-24 hours
3.	<i>Staphylococcus aureus</i> ATCC 29737	50 -100	good-luxuriant	Amikacin, Chlortetracycline, Cycloserine, Demeclocycline, Kanamycin, Methacycline, Oxytetracycline, Rolitetracycline, Lincomycin, Tetracycline, Tobramycin, Doxycycline	32-35°C / 24 hours
4.	<i>Enterococcus hirae</i> ATCC 10541	50 -100	good-luxuriant	Gramicidin	36-37.5°C / 16-18 hours
5.	<i>Staphylococcus aureus</i> ATCC 9144	50 -100	good-luxuriant	Tylosin	35-39°C / 16-18 hours

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Preparation of Stock cultures

1. Maintain stock cultures on agar slants and make transfers at 1- or 2-week intervals.
2. Using sterile purified water, saline or Antibiotic Medium No.3, prepare the inoculum for assay by washing growth from a fresh 24-48 hour agar slant and further dilute the culture to obtain the desired organism concentration.
3. In some turbidimetric assays, an 18- 24hour culture of the test organism grown in Antibiotic Medium No. 3, diluted to obtain the optimal number of organisms, is used.



Turbidimetric Assay

1. Use glass or plastic test tubes (i.e., 16 × 125 mm or 18 × 150 mm) that are relatively uniform in length, diameter and thickness and substantially free from surface blemishes.
2. Tubes that will be placed in the spectrophotometer should be matched and free of scratches or blemishes.
3. Clean the tubes thoroughly to remove all antibiotic residues and traces of cleaning solution and, prior to subsequent use, sterilize tubes that have been previously used.
4. Prepare working dilutions of the antibiotic reference standards in specific concentrations.
5. To a 1 mL quantity of each solution in a suitable tube, add 9 mL of inoculated broth, as required.
6. Prepare similar solutions of the assay materials containing approximately the same amounts of antibiotic activity and place in tubes.
7. 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.
8. Determine the amount of growth by measuring light transmittance with a suitable spectrophotometer.
9. Determine the concentration of the antibiotic by comparing the growth obtained with that given by reference standard solutions.
10. Refer to appropriate procedures outlined in the references for a complete discussion of antibiotic assay methods.

Results

Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Antibiotic Assay Medium No.3

Product Code : DMO16U

Available Pack sizes : 100gm/ 500gm

References

1. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
2. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/ The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
3. Rippere R. A.. Some principles of microbiological turbidimetric assays of antibiotics. J. Assoc. off. Anal. Chem. 1979. 62(4):951-6.
4. United States Pharmacopoeia 2011, US Pharmacopoeial Convention, Inc., Rockville, MD.

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Further Information

For further information please contact your local MICROMASTER Representative.



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