



# PRODUCT SPECIFICATION SHEET

## Antibiotic Assay Medium No.8 (DM020U)

### Intended Use

Antibiotic Assay Medium No. 8 (DM020U) is recommended for microbiological assay of Mitomycin, Plicamycin and Vancomycin, in compliance with USP.

### Product Summary and Explanation

Antibiotic assay media are prepared according to the specifications of the USP<sup>(1)</sup>, European Pharmacopeia<sup>(2)</sup> and AOAC International.<sup>(3)</sup> The antibiotic media are identified numerically with names assigned by Grove and Randall in *Assay Methods of Antibiotics*.<sup>(4)</sup> The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.<sup>(1)</sup> An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganisms. Biological tests offer the most convenient means of performing an assay,<sup>(5)</sup> since a reduction in the antimicrobial activity of a specific antibiotic reveals changes not usually displayed by chemical methods.<sup>(6)</sup> Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al.<sup>(7)</sup> for the assay of penicillin, was later modified by Foster and Woodruff<sup>(8)</sup> and by Schmidt and Moyer.<sup>(9)</sup> The choice of methodology is often based on many factors, including relative ease of performance, flexibility and use of automated or semi-automated devices for both identification and susceptibility testing.<sup>(10)</sup>

Antibiotic Assay Medium No. 8 is commonly used as base agar for microbiological agar diffusion assays for wide variety of antibiotics. This medium is prepared according to the specifications detailed in the USP and CFR.<sup>(11,12)</sup>

### Principles of the Procedure

Antibiotic Assay Medium No. 8 contains peptone, yeast extract and beef extract which provides nitrogenous growth factors, vitamins and other essential growth nutrients. This medium provides solidified substratum for growth of organisms. This medium provides the optimal pH 5.9 for assay of tetracycline as these antibiotics are stable at slightly lower pH.<sup>(13)</sup> This pH condition also supports the growth of test organisms. This medium is also used as base and seed agar medium for agar diffusion assay for mitomycin, mithramycin, plicamycin and Vancomycin.<sup>(14)</sup>

### Cylinder Plate Assay

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

Prediffusion of antibiotics for 10-20 mins in the agar by incubating at temperature below the optimal growth temperature for microorganism would facilitate better diffusion of antibiotics followed by incubation of plates for microbial growth.

### Note:

Recommended for the Microbiological assay of Vancomycin.

### Formula / Liter

Ingredients	Gms / Liter
Peptone	6.00
Yeast extract	3.00
Beef extract	1.50
Agar	15.00
Final pH: 5.9 ± 0.1 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.



## PRODUCT SPECIFICATION SHEET

- Freshly prepared plates should be used for antibiotic assays.
- All conditions in the microbiological assay must be controlled carefully.
- The use of standard culture medium in the test is one of the important steps for obtaining good results.

### Directions

- Suspend 25.5 grams of the medium in one liter of purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

### Quality Control Specifications

<b>Dehydrated Appearance</b>	Cream to yellow coloured homogeneous free flowing powder
<b>Prepared Medium</b>	Light amber coloured slightly opalescent gel forms in Petri plates
<b>Reaction of % solution</b>	Not Applicable
<b>Gel Strength</b>	Firm, comparable with 1.5% Agar gel

### Growth Promotion Test

As per United States Pharmacopoeia.

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 32 - 35°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Antibiotics Assayed
1.	<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant	≥70%	Vancomycin

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

### Test Procedure

#### Preparation of Stock cultures

- Maintain stock cultures on agar slants and make transfers at 1- or 2-week intervals.
- 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.
- For using as a test organism *Bacillus subtilis*, inoculate the organism on Antibiotic Medium No. 1 and incubate at 37°C for 1 week. Wash spores from the agar surface, and heat the spores at 56°C for 30 minutes. Using sterile purified water wash the spores three times, heat again at 65°C for 30 minutes, and then dilute to the optimal concentration. Inoculum prepared following this method should produce a sharp zone in the assay.
- For preparing spore suspension of *B. Subtilis*, Antibiotic Medium No. 1 modified by the addition of 300 mg manganese sulfate ( $MnSO_4 \cdot H_2O$ ) per liter is used which aids in the sporulation of *B. Subtilis*.

#### 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 ( $\pm 0.1$  mm): 8 mm outside diameter, 6 mm inside diameter and 10 mm long. Clean the cylinders carefully to remove all residues, using an occasional acid bath (i.e., with approximately 2N nitric acid or with chromic acid).
- Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius.
- For assuring accurate assays, use a level surface for working 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 2.1 mL base layer and a 4 mL seed layer.



## PRODUCT SPECIFICATION SHEET

6. 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.

### Results

1. After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic.
2. Refer to appropriate references and specific test procedures.

### 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.8**

**Product Code : DM020U**

**Available Pack sizes : 500gm**

### References

1. United States Pharmacopoeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopoeial Convention, Inc., Rockville, Md.
2. Council of Europe. 2002. European pharmacopeia, 4th ed. Council of Europe, Strasbourg, France.
3. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
4. Grove and Randall. 1955. Assay methods of antibiotics. Medical Encyclopedia, Inc. New York, N.Y.
5. Pelczar M. J. Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Ed, Tata McGraw-Hill Publishing Company Ltd, New Delhi.
6. The United States Pharmacopoeia, 2009, The United States Pharmacopoeial Convention, Rockville, MD.
7. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings. 1941. Lancett ii:177.
8. Foster and Woodruff. 1943. J. Bacteriol. 46:187.
9. Schmidt and Moyer. 1944. J. Bacteriol. 47:199.
10. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
11. United States Pharmacopoeia / National Formulary 2011, US Pharmacopoeial Convention, Inc., Rockville, MD.
12. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436, Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242-259, (April 1).
13. Chapin-Robertson and Edberg, 1991, Measurement of Antibiotics in Human Body fluids: Techniques and significance. Antibiotics in Laboratory medicine, New York 311.
14. United States Pharmacopoeia/National Formulary (USP21/NF16) 1985, US Pharmacopoeial Convention, Inc., Rockville, MD.





# PRODUCT SPECIFICATION SHEET

---

## Further Information

For further information please contact your local MICROMASTER Representative.



### MICROMASTER LABORATORIES PRIVATE LIMITED

DM020UPSS, QAD/FR/024, Rev.00

Unit 38/39, Kalpataru Industrial Estate,  
Off G. B. Road, Near 'R-Mall', Thane (W) - 400607. M.S. INDIA.  
Ph: +91-9320126789/9833630009/9819991103  
Email: [sales@micromasterlab.com](mailto:sales@micromasterlab.com)

#### Disclaimer :

All Products conform exclusively to the information contained in this and other related Micromaster Publications. Users must ensure that the product(s) is appropriate for their application, prior to use. The information published in this publication is based on research and development work carried out in our laboratory and is to the best of our knowledge true and accurate. Micromaster Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are intended for laboratory, diagnostic, research or further manufacturing use only and not for human or animal or therapeutic use, unless otherwise specified. Statements included herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

