



# PRODUCT SPECIFICATION SHEET

## Cetrimide Agar (DM057U)

### Intended Use

Cetrimide Agar (DM057U) is recommended as a selective medium for the isolation of *Pseudomonas aeruginosa* from pharmaceutical products in compliance with USP.

### Product Summary and Explanation

*Pseudomonas aeruginosa* is a gram-negative, aerobic bacterium which grows well on all normal laboratory media but specific isolation of the organism, from environmental sites or from human, animal or plant sources, is best carried out on a medium, which contains a selective agent and also constituents to enhance pigment production. Most selective media depend upon the intrinsic resistance of the species to various antibacterial agents. King et al.<sup>(1)</sup> developed the formulation of Cetrimide Agar. This media is based on the formulation described in USP<sup>(2)</sup> and is in accordance with the harmonized method of USP/EP/BP/JP/IP.<sup>(2,3,4,5,6)</sup> It is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from pharmaceutical products. In 1951, Lowbury<sup>(7)</sup> described the use of 0.1% cetrimide in a selective medium for *P. aeruginosa*. Because of the increased purity of the inhibitory agent, the concentration was later reduced, as reported by Lowbury and Collins in 1955.<sup>(8)</sup>

Cetrimide is a quaternary ammonium salt, which acts as a cationic detergent that reduces surface tension in the point of contact and has precipitant, complexing and denaturing effects on bacterial membrane proteins. It exhibits inhibitory actions on a wide variety of microorganisms including *Pseudomonas* species, whilst allowing *Pseudomonas aeruginosa* to develop typical colonies. Brown and Lowbury employed incubation at 37°C with examination after 18 and 42 hours of incubation.<sup>(9)</sup>

*P.aeruginosa* can be identified due to their characteristic production of pyocyanin, a blue, water-soluble, non-fluorescent phenazine pigment coupled with their colonial morphology and the characteristic grape-like odor of aminoacetophenone.<sup>(10)</sup> *P.aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. These media are therefore, important in the identification of *P.aeruginosa*.

### Principles of the Procedure

Cetrimide Agar contains pancreatic digest of gelatin which supplies the nutrients necessary to support growth of the organisms. The production of pyocyanin a blue-green pigment is stimulated by the magnesium chloride and dipotassium sulfate in the medium, which improves detection of *Pseudomonas* on this medium. Presence of magnesium ions can also neutralize EDTA, if present in the sample. Cetrimide is a quaternary ammonium, cationic detergent compound, which is inhibitory to a wide variety of bacterial species other than *P. aeruginosa*. Cetrimide causes nitrogen and phosphorus to be released from bacterial cells other than *Pseudomonas aeruginosa*. Cetrimide Agar is supplemented with 1% glycerol as a source of carbon for growing cells.

### Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of gelatin	20.00
Magnesium chloride	1.40
Dipotassium sulphate	10.00
Cetrimide	0.30
Agar	13.60
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	





## PRODUCT SPECIFICATION SHEET

### Precautions

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

### Directions

1. Suspend 45.3 grams of medium in one liter of distilled water water containing 10 ml glycerol.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

### Quality Control Specifications

<b>Dehydrated Appearance</b>	Cream to yellow homogeneous free flowing powder
<b>Prepared Medium</b>	Light amber coloured opalescent gel with a slight precipitate forms in Petri plates
<b>Reaction of 4.53% Solution</b>	pH : 7.2 ± 0.2 at 25°C
<b>Gel Strength</b>	Firm, comparable with 1.36% Agar gel

### Growth Promotion Test

Growth Promotion is carried out in accordance with the method of USP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

### Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating  $\leq 100$  cfu (at 30-35°C for  $\leq 18$  hours).

### Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating  $\geq 100$  cfu (at least 100 cfu) (at 30-35°C for  $\geq 72$  hours).

**Expected Cultural Response:** Cultural characteristics observed after incubation at 30-35 °C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation Temperature & Time
	<b>Growth promoting</b>	--	--	--	--	--
1.	<i>Pseudomonas aeruginosa</i> ATCC 9027	50 - 100	good-luxuriant	25 -100	$\geq 50$ %	30-35°C $\leq 18$ hrs
	<b>Inhibitory</b>	--	--	--	--	--
2.	<i>Escherichia coli</i> ATCC 8739	$\geq 10^3$	inhibited	0	0%	30-35°C $\geq 72$ hrs
	<b>Additional Microbiological testing</b>	--	--	--	--	--





## PRODUCT SPECIFICATION SHEET

3.	<i>Pseudomonas aeruginosa</i> ATCC 27853	50 - 100	good-luxuriant	25 - 100	≥50 %	30-35°C 18-24 hrs
4.	<i>Pseudomonas aeruginosa</i> ATCC 25668	50 - 100	good-luxuriant	25 - 100	≥50 %	30-35°C 18-24 hrs
5.	<i>Stenotrophomonas maltophilia</i> ATCC 13637	≥10 <sup>3</sup>	inhibited	0	0%	30-35°C ≥72 hrs
6.	<i>Escherichia coli</i> ATCC 25922	≥10 <sup>3</sup>	inhibited	0	0%	30-35°C ≥72 hrs
7.	<i>Escherichia coli</i> NCTC 9002	≥10 <sup>3</sup>	inhibited	0	0%	30-35°C ≥72 hrs
8.	<i>Staphylococcus aureus</i> ATCC 6538	≥10 <sup>3</sup>	inhibited	0	0%	30-35°C ≥72 hrs
9.	<i>Staphylococcus aureus</i> ATCC 25923	≥10 <sup>3</sup>	inhibited	0	0%	30-35°C ≥72 hrs
10.	<i>Salmonella typhimurium</i> ATCC 14028	≥10 <sup>3</sup>	inhibited	0	0%	30-35°C ≥72 hrs
11.	<i>Proteus mirabilis</i> ATCC 29906	≥10 <sup>3</sup>	inhibited	0	0%	30-35 °C ≥72 hrs

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

### Test Procedure

1. For the isolation of *P.aeruginosa*, plates of Cetrimide Agar should be inoculated from non-selective medium such as Soybean Casein Digest Medium (DM277U).
2. If the count is high, the test sample can be directly inoculated onto Cetrimide Agar.
3. Refer to appropriate references for standard test procedures.

### Results

1. Colonies that are surrounded by a blue-green pigment and fluoresce under short wavelength (254 nm) ultraviolet light may be presumptively identified as *Pseudomonas aeruginosa*.
2. Note, however, that certain strains of *P. aeruginosa* may not produce pyocyanin.
3. Other species of *Pseudomonas* do not produce pyocyanin, but fluoresce under UV light.
4. Most non-*Pseudomonas* species are inhibited, and some species of *Pseudomonas* may also be inhibited.
5. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.
6. Refer to appropriate references and standard 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.





## PRODUCT SPECIFICATION SHEET

### Limitations of the Procedure

1. *Pseudomonas aeruginosa* colonies may appear pigmented greenish (under UV light also). Addition of nalidixic acid can aid in inhibiting the growth of accompanying flora.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

### Packaging

Product Name : Cetrimide Agar

Product Code : DM057U

Available Pack sizes : 100gm / 500gm

### References

1. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
2. The United States Pharmacopoeia, 2011 The United States Pharmacopoeial Convention. Rockville, MD.
3. European Pharmacopoeia, 2011 European Dept. for the quality of Medicines.
4. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
5. Japanese Pharmacopoeia, 2008
6. Indian Pharmacopoeia, 2010, Govt. of India, Ministry of Health and Family Welfare, New Delhi.
7. Lowbury, 1951, J. Clin. Pathol., 4:66.
8. Lowbury and Collins, 1955, J. Clin. Pathol., 8:47
9. Brown and Lowbury, 1965, J. Clin. Pathol., 18:752.
10. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8<sup>th</sup> Ed., American Society for Microbiology, Washington, D.C.

### Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

DM057UPSS,QAD/FR/024,Rev.00/01.01.2018

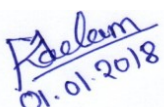
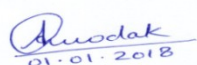

Unit 38/39, Kalpataru Industrial Estate,

Off G.B. Road, Near 'R-Mall', Thane (W) - 400607. M.S. INDIA.

Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.

Email: [micromaster@micromasterlab.com](mailto:micromaster@micromasterlab.com)

[sales@micromasterlab.com](mailto:sales@micromasterlab.com)

	Checked By	Approved By
 01.01.2018	 01.01.2018	 01.01.2018
Microbiologist	Head Quality Control	Head Quality Assurance





## PRODUCT SPECIFICATION SHEET

---

### 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.

