



PRODUCT SPECIFICATION SHEET

Cystine Tryptone Agar (DM068)

Intended Use

Cystine Tryptone Agar (DM068) is recommended for maintenance, subculturing, detection of motility and carbohydrate fermentation studies.

Product Summary and Explanation

Cystine Tryptone Agar is suitable for the propagation and maintenance of bacteria even the fastidious ones without addition of additives. This formulation was developed by Vera as a simple semisolid medium for the identification and maintenance of the *Gonococcus* and other bacteria.⁽¹⁾ Without carbohydrates, it can be used for maintenance of cultures, including fastidious organisms, by a deep stab, like *Brucella*, *Corynebacterium*, *Pasteurella*, *Pneumococci* and *Streptococci* without added enrichments⁽²⁻⁴⁾ for longer periods when stored at appropriate temperatures. Even some light-sensitive anaerobic microorganisms can grow in this medium without special conditions, though in reduced atmospheres, they give ideal growth. This medium has its maximum efficiency when freshly prepared, but if dehydration of the medium is avoided, it can be stored for long period of time. To achieve this, screw caps or proper sealing are strongly recommended. In presence of CO_2 , anaerobic organisms like *Actinomyces bovis*, *Bacteroides funduliformis* and *Leptotrichia*⁽⁵⁾ grow well in this medium. With the appropriate carbohydrate, it is recommended for the differentiation of fastidious organisms that do not grow on phenol red classical media, by means of fermentation reactions. In semisolid agar, acid reactions are easily detected because the acid formed is not immediately diffused throughout the entire culture as in broth. Most cultures show an alkaline reaction when no fermentable carbohydrate is present. Motility can be readily detected in the semisolid medium.⁽⁶⁾

Principles of the Procedure

Cystine Tryptone Agar contains casein enzymic hydrolysate, L-cystine which supplies the nutrients necessary to support the growth of fastidious microorganism. Carbohydrate fermentation is detected by a visible colour change of the medium due to the incorporation of the pH indicator dye, phenol red. The medium becomes acidified due to the production of organic acids, when the organism metabolizes the carbohydrate present. However, the peptones present in the medium are also degraded by the bacteria present and yield substances that are alkaline in pH. The phenol red indicator changes from reddish-orange to yellow when the amount of acid produced by carbohydrate fermentation is greater than the alkaline end products of the peptone degradation. The colour change with phenol red occurs around pH 6.8, near the original pH of the medium.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	20.00
L-Cystine	0.50
Sodium chloride	5.00
Sodium sulphite	0.50
Phenol red	0.017
Agar	2.50
Final pH: 7.3 ± 0.2 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.

Directions





PRODUCT SPECIFICATION SHEET

1. Suspend 28.51 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense in tubes in 8-10 ml amounts.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool to 50°C and add appropriate carbohydrate (0.5 to 1.0% if desired).
6. Mix well and allow the tubed medium to cool in an upright position.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light pink homogeneous free flowing powder
Prepared Medium	Red coloured, clear to slightly opalescent gel forms in tubes as butts
Reaction of 2.85% Solution	pH : 7.3 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 0.25% Agar gel.

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 4-18 hours or longer if necessary.

Sr. No.	Organisms	Inoculum (CFU)	Results to be achieved		
			Growth	Motility	Acid in presence of Dextrose
1.	<i>Escherichia coli</i> ATCC 25922	50 - 100	good-luxuriant	positive, growth away from stabline causing turbidity	positive reaction, yellow colour
2.	<i>Neisseria gonorrhoeae</i> ATCC 19424	50 - 100	Good	negative, growth along the stabline, surrounding medium	positive reaction, yellow colour
3.	<i>Neisseria meningitides</i> ATCC 13090	50 - 100	Good	negative, growth along the stabline, surrounding medium	positive reaction, yellow colour
4.	<i>Streptococcus pneumonia</i> ATCC 6303	50 - 100	Good	negative, growth along the stabline, surrounding medium	positive reaction, yellow colour

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

Test Procedure

1. Only the surface of the tubed medium is inoculated in case of fermentation studies of the genus *Neisseria*.
2. For facultative organisms, such as *Streptococci* and strictly anaerobic organisms inoculation is done by stabbing the center of the medium with an inoculating needle to about half the depth of the medium.
3. Incubate with loosened caps aerobically or anaerobically depending upon the organisms being tested.
4. *Neisseria* should be incubated with loose caps; if incubated in CO₂ incubator or with tight caps in non-CO₂ incubator.
5. For more rapid growth and also for more rapid fermentation reactions, anaerobic cultures preferably should be incubated in the presence of CO₂ as well as hydrogen or nitrogen. Some strict anaerobes fail to grow or grow poorly in the absence of CO₂.

Results

1. A yellow colour either in the upper one-third or throughout the medium indicates acid production due to carbohydrate fermentation.
2. A red (alkaline) to orange (neutral) colour indicates that the carbohydrate has not been degraded and that only the peptone has been utilized.
3. Inoculated medium (without carbohydrate) also exhibits a red to orange colour.





PRODUCT SPECIFICATION SHEET

4. Motile cultures show growth away from the line of inoculation.
5. Non-motile organisms grow in the inoculated area, along the stab line while the surrounding area remains clear.

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. Cystine Tryptone Agar requires a heavy inoculum.
2. Prolonged incubation may lead to changes in pH indicator or abnormal lactose/sucrose reactions with *Neisseria* pathogens.
3. *Neisseria* species usually produce acid only in the area of stabs (upper third). If there is a strong acid (yellow color) throughout the medium, a contaminating organism may be present.
4. Gram stain and oxidase test should be performed on the growth to confirm the presence of *Neisseria* species.
5. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Cystine Tryptone Agar

Product Code : DM068

Available Pack sizes : 100gm/ 500gm

References

1. Vera H. D., 1948, J. Bacteriol. 55:531.
2. Peterson and Hartsell, 1955, J. Inf. Dis., 96:75.
3. Myers and Koshy, 1962, Am. J. Public Health, 96:75.
4. Alford, Wiese and Gunter, 1955, J. Bacteriol. 69:518.
5. Kroeger and Sibal, 1961, J. Bacteriol. 50:581.
6. Vera and Petran, 1954, Bull. Nat. Assoc. Clin. Labs., 5:90

Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

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

DM068PSS,QAD/FR/024,Rev.00





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.

