



PRODUCT SPECIFICATION SHEET

Lactose TTC Agar with Tergitol™ -7 (DM833)

Intended Use

Lactose TTC Agar with Tergitol-7 (DM833) is recommended for selective enumeration and identification of coliforms.

Product Summary and Explanation

Lactose TTC Agar with Tergitol-7 is a selective and differential medium for the detection and enumeration of coliforms in food and water samples. Lactose TTC Agar with Tergitol-7 is based on the formulation described by Chapman⁽¹⁾ and is recommended for the selective isolation and differentiation of the coliform group. Chapman reported that the addition of Tergitol-7 to a medium consisting of polypeptone and yeast extract permitted unrestricted development of all coliform bacteria, and inhibited gram-negative spore-formers and Gram-positive organisms. Coliform counts on Tergitol-7 Agar were 30% higher than on other selective media. Chapman modified his original Tergitol-7 Agar by adding triphenyl tetrazolium chloride (TTC) to the original formula. The addition of tri-phenyltetrazolium chloride (TTC) allows earlier recognition and identification of *Escherichia coli* and *Enterobacter aerogenes*. Confirmation of the presence of *E. coli* was possible after only 10 hours incubation at 35°C. Chapman also reported that Tergitol-7 Agar with added TTC gave a selective medium suitable for the isolation of *Candida* spp. and other fungi. Tergitol-7 inhibits Gram positive organisms and minimises the swarming of *Proteus* allowing superior recovery of coliforms. TTC is rapidly reduced to insoluble red formazan by most coliform organisms except *E. coli* and *Enterobacter aerogenes*, thus allowing easy differentiation. Pollard⁽²⁾ has reported the selective bactericidal property of sodium heptadecyl sulphate (Tergitol-7). Kulp et. al⁽³⁾ corroborated the use of Tergitol-7 Agar with TTC in routine analysis of water and Mossel used this medium for the examination of food materials.

Principles of the Procedure

Lactose TTC Agar with Tergitol-7 contains proteose peptone serves as sources of carbon, nitrogen and other essential growth nutrients. Yeast extract provides vitamin B complex required for growth. Lactose is the fermentable carbohydrate. Lactose fermentation is indicated by a color change of the pH indicator, Bromthymol Blue. Selectivity is achieved by the use of sodium heptadecylsulfate (Tergitol 7) and Triphenyltetrazoliumchloride (TTC) to inhibit most Gram-positive bacteria and *Proteus* swarming and yields better recovery of coliforms. TTC is also part of the differential system. The reduction of TTC by lactose - negative bacteria produces dark red colonies. Lactose - positive *E.coli* and coliform bacteria reduce TTC weakly; hence their colonies are yellow- orange.

Formula / Liter

Ingredients	Gms / Liter
Peptone	10.00
Yeast extract	6.00
Lactose	20.00
Bromo thymol blue	0.05
Sodium heptadecyl sulphate(Tergitol-7)	0.10
Agar-agar	12.70
Final pH: 7.2 ± 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

1. Suspend 53.9 grams of the medium in one liter of distilled water.



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- Heat to boiling, to dissolve the medium completely.
- Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
- Cool to 45-50°C.
- Aseptically add 50 ml of sterile filtrated 0.05 % aqueous solution of Triphenyl Tetrazolium Chloride (TTC) Solution to one liter of media.
- Mix well and pour into sterile Petri plates. The agar layer should have a height of at least 5 mm.

Quality Control Specifications

Dehydrated Appearance	Cream to light green homogeneous free flowing powder
Prepared Medium	Green coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 3.31% solution	pH 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.27% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours, with added TTC solution 1% (MS029).

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of colony
1.	<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	≥50%	Yellow - Orange colonies with yellow zone
2.	<i>Citrobacter freundii</i> ATCC 8090	50-100	good-luxuriant	≥50%	Yellow - Orange colonies with yellow zone
3.	<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	good-luxuriant	40-50%	Dark Red with blue zone
4.	<i>Bacillus cereus</i> ATCC 11778	≥10 ³	inhibited	0%	--

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

- Lactose fermenting organisms form yellow colonies with yellow zones. Lactose non-fermenters produce Red colonies with blue zone.
- TTC is reduced by the bacterial cell except *Escherichia coli* and *Enterobacter aerogenes* to form formazan, a red coloured insoluble complex, thereby producing red coloured colonies.
- 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.

The TTC- solution and the prepared medium is stable for 4 week when stored at +2 °- + 8 °C and protected from 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.



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Limitations of the Procedure

1. Pour plates do not give satisfactory results.
2. Allow plates to dry with lids slightly ajar for 1 - 2 hours.
3. Reduction of TTC is an irreversible reaction that produces an insoluble formazan compound.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Lactose TTC Agar with Tergitol-7

Product Code : DM833

Available Pack sizes : 500gm

References

1. Chapman, G. H. 1947. A superior culture medium for the enumeration and differentiation of coliforms. J. Bacteriol. 53:504.
2. Pollard A.L., 1946, Science, 103:758.
3. Kulp W., Mascoli C., Tavshanjian O. (1953) Am. J. Pub. Hlth 43. 1111-1113.

Further Information

For further information please contact your local MICROMASTER Representative.



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