PRODUCT SPECIFICATION SHEET

DNase Test Agar w/ Toluidine Blue (DM301)

Intended Use
DNase Test Agar w/Toluidine Blue (DM301) is recommended for detection of deoxyribonuclease activity of microorganisms.

Product Summary and Explanation
The DNase test is used to detect the degradation of deoxyribonucleic acid (DNA). The test is useful for differentiating *Serratia* from *Enterobacter*, *Staphylococcus aureus* from coagulase-negative staphylococci, and *Moraxella catarrhalis* from *Neisseria* species.[1, 2] In 1957, Jeffries et al. described a rapid agar plate method for demonstrating DNase activity of microorganisms.[3] This procedure utilized a semi-synthetic medium with nucleic acid solution incorporated in the medium. Enzymatic activity is detected by flooding the plate with 1 N hydrochloric acid (HCl). A clear zone surrounding growth indicates a positive reaction.[4]
DNase activity was observed by Weckman and Catlin[5] in Micrococci and found the correlation with coagulase activity as coagulase positive species were DNase positive. Di Salvo[6] confirmed the results of Weckman and Catlin and observed accurate correlation of DNase and coagulase activity. In his experiment Di Salvo incorporated DNA and calcium chloride to activate DNase enzyme. Schreier modified DNase medium by adding toluidine blue.[7] Modified medium achieved faster identification of Serratia marcescens and could differentiate Serratia from other members of the Enterobacteriaceae. DNase Test Agar with Toluidine Blue contains a metachromatic dye to eliminate the necessity of reagent addition to the agar following incubation.[8] Toluidine blue may be toxic to some gram-positive cocci and, therefore, should be used primarily with *Enterobacteriaceae*.

Principles of the Procedure
DNase Test Agar w/ Toluidine Blue contains tryptose which provides amino acids and other complex nitrogenous substances to support bacterial growth. Sodium chloride helps in maintaining the osmotic equilibrium. DNA is the substrate for DNase activity. DNase is an extracellular enzyme that breaks the DNA down into subunits composed of nucleotides. DNase depolymerizes the DNA resulting in the formation of a clear zone around the microbial growth which is visualized by flooding the plate with hydrochloric acid
The HCl reagent is not needed to detect DNase activity on DNase Agar with Toluidine Blue. Toluidine blue forms a complex with intact (polymerized) DNA. In the intact DNA complex, the toluidine blue has the normal blue color. DNase activity depolymerizes the DNA, breaking down the dye-DNA complex. In the presence of nucleotides produced from the DNase depolymerization, the dye takes on its metachromatic color, forming pink to red zones around bacterial growth. A negative test is indicated when the medium remains blue.

Formula / Liter

Ingredients	Gms / Liter
Tryptose	20.00
Deoxyribonucleic acid (DNA)	2.00
Sodium chloride	5.00
Toluidine blue	0.10
Agar	15.00
Final pH: 7.3 ± 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 42.10 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely, with frequent agitation.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well, cool to 45°C and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light blue homogeneous free flowing powder
Prepared Medium	Blue coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 4.2% solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Serratia marcescens ATCC 8100*	50-100	good-luxuriant	positive reaction, pink to red zone around the growth
2.	*Staphylococcus aureus ATCC 25923*	50-100	good-luxuriant	positive reaction, pink to red zone around the growth
3.	*Staphylococcus epidermidis ATCC 12228*	50-100	good-luxuriant	negative reaction
4.	*Streptococcus pyogenes ATCC 19615*	50-100	good-luxuriant	positive reaction, pink to red zone around the growth

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

Test Procedure

1. Inoculate by making a single streak line using inoculum from an agar slant or plate.
2. Incubate at 35 ± 2°C for 24-48 hours. Plates should be incubated in an inverted position.

Results

1. DNase activity results in the production of a bright pink reaction due to the metachromatic property of toluidine blue, where a pink to red halo is observed surrounding the growth and the rest of the plate remains blue.
2. Some strains of Staphylococci may be inhibited on DNase Test Agar due to toluidine blue.
3. Further confirmatory tests for the identification should be carried out.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

PRODUCT SPECIFICATION SHEET

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 : DNase Test Agar w/ Toluidine Blue
Product Code : DM301
Available Pack sizes : 100gm / 500gm

References

1. Washington. 1985. Laboratory procedures in clinical microbiology, 2nd ed. Springer-Verlag, New York, N.Y.
2. Ma cFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
3. Jeffries, Holtman and Guse. 1957. J. Bacteriol. *73*:590.
4. Streitfeld, Hoffman and Janklow, 1962, J. Bact., 84:77.
5. Weckman and Catlin, 1957, J. Bact., 73:747.
6. Di Salvo, 1958, Med. Tech. Bull., U.S. Armed Forces Med. J., 9:191.
7. Schreir, 1969, Am. J. Clin. Pathol., 51:711.
8. Smith, Hancock and Rhoden. 1969. Appl. Microbiol. *18*:991.

Further Information

For further information please contact your local MICROMASTER Representative.

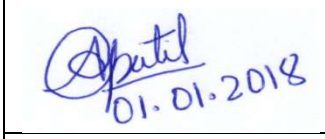
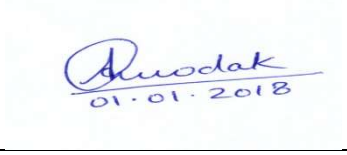
MICROMASTER LABORATORIES PRIVATE LIMITED

Unit 38/39, Kalpataru Industrial Estate,
Near Runwal Estate, Behind 'R-Mall' ,Ghodbunder Raod,
Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

QAD/FR/024,Rev.00/01.01.2018

PRODUCT SPECIFICATION SHEET

S

Prepared By	Checked By	Approved By
		
Microbiologist	**Head Quality Control**	**Head Quality Assurance**

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.