PRODUCT SPECIFICATION SHEET

DNase Test Agar Base (DM075)

Intended Use
DNase Test Agar Base (DM075) is recommended for detection of deoxyribonuclease activity of bacteria and fungi, and especially for identification of pathogenic *Staphylococci*.

Product Summary and Explanation
DNase Test Agar is used for detecting deoxyribonuclease activity of bacteria and fungi and particularly for identification of pathogenic Staphylococci. With added toluidine blue, it is used in differentiation and identification of nonpigmented *Serratia* species isolated from clinical sources that might be improperly identified as *Enterobacter* and In 1956, Weckman and Catlin showed a correlation between increased DNase activity of *Staphylococcus aureus* and positive coagulase activity.[1] Their research suggested DNase activity could be used to identify potentially pathogenic staphylococci In 1957, Jeffries et al. described a rapid agar plate method for demonstrating DNase activity of microorganisms.[2] 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. DiSalvo[3] confirmed the correlation between coagulase activity and DNase activity by incorporating DNA into the medium along with calcium chloride to activate the enzyme. Di Salvo incorporated DNA and calcium chloride to activate DNase enzyme. Schreier modified DNase medium by adding toluidine blue.[4] This modified medium achieved faster identification of *Serratia marcescens* and could differentiate *Serratia* from other members of the *Enterobacteriaceae*.

Principles of the Procedure
DNase Test Agar Base contains casein enzymic hydrolysate, papaic digest of soyabean meal which provides amino acids and other complex nitrogenous substances to support bacterial growth. Sodium chloride helps to maintain the osmotic balance of the medium. The dye (toluidine blue) forms a complex with the DNA present in the medium. The complex thus formed helps the dye to retain its original colour. As soon as the DNA (in the complex) is hydrolysed by DNase of the test organisms, the complex is broken down and colourless zones are formed around the colonies. This can be visualized by flooding the plate with hydrochloric acid.[5] However, in case of toluidine blue, the nucleotides formed due to DNA depolymerization, helps the dye to take its metachromatic colour and in the process forming pink to red zones around the colonies.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	15.00
Papaic digest of soyabean meal	5.00
Deoxyribonucleic acid (DNA)	2.00
Sodium chloride	5.00
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	

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

Directions
1. Suspend 42 grams of medium in one liter of distilled water.
2. Heat with frequent agitation to dissolve the medium completely.
3. Autoclave at 118°C to 121°C, 12 to 15 psi pressure, for 15 minutes.
4. Cool to 45°C and pour into sterile petriplates.

5. Add 0.1 gm Toluidine Blue (MS151) before sterilizing the medium or flood the plates with 0.1% Toluidine Blue (MS151) solution after incubation as desired.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light pink homogeneous free flowing powder
Prepared Medium	Basal medium :Light amber; After addition of Toluidine blue(MS151) : Blue coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 4.0% Solution	pH : 7.3±0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% agar gel.

Expected Cultural Response: Cultural characteristics observed with added Toluidine Blue (FD051) after an incubation at 35 - 37°C for 18 – 24 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	DNase Activity
1.	*Serratia marcescens* ATCC 8100	50-100	good-luxuriant	positive, change in colour from blue to pink purple around the growth when toluidine blue is used / clear one surrounding colonies when plates w/1N HCL are flooded
2.	*Staphylococcus aureus* ATCC 25923	50-100	good-luxuriant	positive, change in colour from blue to pink purple around the growth when toluidine blue is used / clear one surrounding colonies when plates w/1N HCL are flooded
3.	*Staphylococcus epidermidis* ATCC 12228	50-100	good-luxuriant	negative reaction
4.	*Streptococcus pyogenes* ATCC 19615	50-100	good-luxuriant	positive, change in colour from blue to pink purple around the growth when toluidine blue is used / clear one surrounding colonies when plates w/1N HCL are flooded

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. One plate may be inoculated with up to eight isolates by spot inoculation (1/8 to 1/4 inch) or streak inoculation (a single 1- to 2-inch line).
2. Incubate at 35 ± 2°C for 24-48 hours. Plates should be incubated in an inverted position. Incubate tubes with loosened caps.
3. Following incubation, flood DNase Test Agar plates with 1N HCl reagent and observe for reaction.
4. Refer to appropriate references for standard test procedures.

Results

1. A change in colour from blue to pink purple around the growth when toluidine blue is used and clear area surrounding growth (band/spot inocula) on DNase Test Agar after the addition of 1N HCl indicates a positive reaction, DNase activity.
2. A negative reaction is indicated by no clearing and a cloudy precipitate around colonies and throughout medium due to precipitated salts in the medium.
3. Refer to appropriate references and 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.

Limitations of the Procedure

1. Some strains of Staphylococci may be inhibited on DNase Test Agar due to toluidine blue. Further confirmatory tests for the identification should be carried out.
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 : DNase Test Agar Base
Product Code : DM075
Available Pack sizes : 100gm/500gm

References

1. Weckman and Catlin, 1957, J. Bact., 73:747.
2. Jeffries C. D., Holtman F., and Guse D. G., 1957, J. Bacteriol., 73:590.
3. Di Salvo, 1958, Med. Tech. Bull., U.S. Armed Forces Med. J., 9:191.
4. Schreir, 1969, Am. J. Clin. Pathol., 51:711.
5. Streitfeld, Hoffman and Janklow, 1962, J. Bact., 84:77.

Further Information

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