DNase Test Agar Base

M482

DNase Test Agar Base is recommended for the detection of deoxyribonuclease activity of bacteria and fungi, and especially for identification of pathogenic Staphylococci.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Deoxyribonucleic acid (DNA)	2.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42 grams in 1000 ml distilled water. Heat with frequent agitation to dissolve the medium completely. Sterilize by autoclaving at 12 to 15 lbs pressure (118°C to 121°C) for 15 minutes. Cool to 45°C and pour into sterile petriplates. Add 0.1 gm Toluidine Blue (FD051) before sterilizing the medium or flood the plates with 0.1% Toluidine Blue (FD051) solution after incubation as desired.

Principle And Interpretation

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 *Klebsiella* species. The correlation between DNase activity and coagulase activity was first studied by Weckman and Catlin (1). Jeffries et al demonstrated DNase activity by the agar plate method employing a semi-synthetic medium (5). Positive DNase activity was visualized as clear zones (around colonies) when the plates were flooded with 1 N hydrochloric acid. DiSalvo (2) 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 by (3). This modified medium achieved faster identification of *Serratia marcescens* and could differentiate *Serratia* from other members of the *Enterobacteriaceae* .

Casein enzymic hydrolysate, papaic digest of soyabean meal provide essential nutrients. The dye (toluidine blue) form 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 (4). 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. 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.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal medium :Light amber ; After addition of Toluidine blue(FD051) : Blue coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.2% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M482: Cultural characteristics observed with added Toluidine Blue (FD051) after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth	DNase Activity
Serratia marcescens ATCC 8100	50-100	luxuriant	positive, change in colour from blue to pink purple around the growth when toluidine blue is used / clear zone surrounding colonies when plates are flooded w/1N HCL
Staphylococcus aureus ATCC 25923	50-100	luxuriant	positive, change in colour from blue to pink purple around the growth when toluidine blue is used/ clear zone surrounding colonies when plates are flooded w/1N HCL
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	positive, change in colour from blue to pink purple around the growth when toluidine blue is used / clear zone surrounding colonies when plates are flooded w/1N HCL

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

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

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