Technical Information

DNase Test Agar Base

Product Code: DM 1482

Application: - 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

Principle & Interpretation

DNase Test Agar is used for detecting deoxyribonuclease activity of bacteria and fungi and specialy 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. Weckman and Catlin [(1)] first studied the correlation between DNase and coagulase activity. Jeffries et al demonstrated DNase activity by the agar plate method using 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. Schreier modified DNase medium by adding toluidine blue by [(3)]. This modified medium helped 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.

Methodology

Suspend 42 grams of powder media in 1000 ml distilled water. Shake well & 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 (MS2051) before sterilizing the medium or flood the plates with 0.1% Toluidine Blue (MS2051) solution after incubation as desired.

Quality Control

Physical 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(MS2051) : 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 range 7.10-7.50

Cultural Response/ characteristices

DM 1482: Cultural characteristics observed with added Toluidine Blue (MS2051) after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth
Serratia marcescens ATCC 8100	50-100	luxuriant
Staphylococcus aureus ATCC 25923	50-100	luxuriant
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant
Streptococcus pyogenes ATCC 19615	50-100	luxuriant

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

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.

Jeffries C. D., Holtman F., and Guse D. G., 1957, J. Bacteriol., 73:590

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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