Technical Information

DNase Test Agar Base w/ methyl green

Product Code: DM 2419

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

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Deoxyribonucleic acid (DNA)	2.000
Sodium chloride	5.000
Methyl green	0.050
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 especially for identification of pathogenic Staphylococci [1]. DNase producing organisms shows clear zone around growth against green background. Reagent addition is not required [2]. This medium is based on modification of the procedure for detecting DNase-producing bacteria as per Smith, Hanoch, and Rhoden [4] and Jefferies, Holtman and Guse [3]. The medium supports growth of both gram positive and gram-negative bacteria.

Tryptose serves as nitrogenous source for the organisms. DNase produced by microorganisms depolymerizes the DNA substrate in the medium. Methyl green fades into a colourless compound producing distinct clear zones surrounding colonies (or band/ spot inocula) in an otherwise green coloured medium. Methyl green requires a highly polymerized DNA substrate [5] and it combines with polymerized DNA forming a stable, green complex at pH 7.5 [6, 7, 8]. As hydrolysis progresses, methyl green is released and when not combined at this pH it fades and becomes a colourless compound. Therefore clear zones are observed [7, 9].

Methodology

Suspend 42.05 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance
Light yellow to greeenish yellow homogeneous free flowing powder.

Gelling
Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Green 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/characteristics
DM 2419: Cultural characteristics observed 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, clear halo around the growth.
Staphylococcus aureus ATCC 25923	50-100	luxuriant	Positive, clear halo around the growth.
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	Negative reaction.
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	Positive, clear halo around the growth.

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. Schreier 1969. Am. J. Clin. Pathol. 51:711.
2. Smith, P.B., Hancock, G. A., and Rhoden, D. L (1969) Appl. Microbiol., 18,991.
3. Jeffries C.D.; Holtman, D.F.; and Guse, D.G (1957) J. Bacteriol., 73, 590.
4. Lachica, R.V.F. and Deibel, R. H (1969). Appl. Environ, Microbiol., 32 (4), 633.
5. Kurnick, N.B (1947). Cold Spring Harbor Symp. Quant. Biol., 12, 141.
6. Kurnick, N.B (1950) Arch. Biochem., 29, 41.
7. Kurnick, N.B and Foster, M. (1950) J. Gen. Microbiol. 33. 243.
8. Kurnick, N.B and Foster, M. (1950) J. Gen. Physiol. 34, 147.

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