

Technical Data

DNase Test Agar w/ Methyl Green

M1419

Intended Use:

Recommended for detection of deoxyribonuclease activity of bacteria and fungi, and especially for identification of pathogenic Staphylococci from clinical samples.

Composition**

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Ingredients	\mathbf{g}/\mathbf{L}
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

Directions

Suspend 42.05 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

DNase test Agar is used for detecting deoxyribonuclease activity of bacteria and fungi and particularly for identification of pathogenic Staphylococci (1). DNase producing organisms exhibit 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 (3) and Jefferies, Holtman and Guse (4). 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).

Type of specimen

Isolated Microorganism from clinical and non clinical samples

Specimen Collection and Handling:

For isolated microorganism samples, follow appropriate techniques for sample collection and processing as per guidelines (1,3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro Diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:

1. The test organisms must be in pure culture and 18-24 hours old.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

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Quality Control

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

pН

7.10-7.50

Cultural Response

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

Organism	Growth	DNase Activity
Serratia marcescens ATCC 8100	luxuriant	positive, clear halo around the growth.
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	luxuriant	positive, clear halo around the growth.
Staphylococcus epidermidis ATCC 12228 (00036*)	luxuriant	negative reaction
Streptococcus pyogenes ATCC 19615	luxuriant	positive, clear halo around the growth.

Key: *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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In vitro diagnostic medical device



Storage temperature



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Do not use if package is damaged

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