Ready Prepared Media

Technical Information

DNase Test Agar Plate w/ Methyl Green

Product Code: PM 2419

Application: Recommended for 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 particularly for identification of pathogenic Staphylococci (9). DNase producing organisms exhibit clear zone around growth against green background. Reagent addition is not required (10). This medium is based on modification of the procedure for detecting DNase-producing bacteria as per Smith, Hanoch, and Rhoden (2) and Jefferies, Holtman and Guse (11). 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 (8) and it combines with polymerized DNA forming a stable, green complex at pH 7.5 (4,5,6). 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 (5,7).

Type of specimen

Isolated microorganisms from food samples

Specimen Collection and Handling

For isolated microorganisms samples, follow appropriate techniques for sample collection and processing as perguidelines (2,11). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. 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.

Methodology

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Quality Control

Appearance
Sterile DNASE Test Agar with Methyl Green in 90mm disposable plates.
Colour
Greenish blue coloured medium
Quantity
25 ml of medium in disposable plate
pH
7.10-7.50
Sterility Check

Passes release criteria

Cultural Response

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

Organism	Inoculum(CFU)	Growth	Dnase Activity
Serratia marcescens ATCC8100	50-100	Luxuriant	Positive,clear halo around the growth.
Staphylococcus aureus subsp. aureus ATCC 25923(00034*)	50-100	Luxuriant	Positive,clear halo around the growth.
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	Luxuriant	Negative reaction.
Streptococcus pyogenes ATCC 19615	50-100	Luxuriant	Positive,clear halo around the growth.

(*) - Corresponding WDCM numbers

Storage and Shelf Life

- On receipt store between 2-8°C Use before expiry date on the label.
- Product performance is best if used within stated expiry period.

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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 samplemust be decontaminated and disposed of in accordance with current laboratory techniques (1,3).

Further Reading

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jeffries C.D.; Holtman, D.F.; and Guse, D.G (1957) J. Bacteriol., 73, 590.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Kurnick, N.B (1947). Cold Spring Harbor Symp. Quant. Biol., 12, 141.
5. Kurnick, N.B (1950) Arch. Biochem., 29, 41.
6. Kurnick, N.B and Foster, M. (1950) J. Gen. Microbiol. 33. 243.
7. Kurnick, N.B and Foster, M. (1950) J. Gen. Physiol. 34, 147.
8. Lachica, R.V.F. and Deibel, R. H (1969). Appl. Environ, Microbiol., 32 (4), 633.
9. Macfaddin, J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Volume1Williams, Wilkins, Baltimore.
10. Schreier 1969. Am. J. Clin. Pathol. 51:711.
11. Smith, P.B., Hancock, G. A., and Rhoden, D. L (1969) Appl. Microbiol., 18,991.

Disclaimer

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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