

Technical Information

DNase Test Agar Base w/o DNA

Product Code: DM 1741

Application: - DNase Test Agar Base with DNA Supplement is recommended for the detection of deoxyribonuclease activity of bacteria and fungi particularly Staphylococci.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
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 Base is used for detecting deoxyribonuclease activity of bacteria and fungi pertaining to 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 mis identified as Enterobacter and Klebsiella species. DNase activity was observed by Weckman and Catlin ⁽¹⁾ in Micrococci and found the correlation between as coagulase positive species & DNase positive strains of s. aureus. Di Salvo ⁽²⁾ confirmed the results of Weckman and Catlin and observed accurate correlation of DNase and coagulase activity. In his experiment Di Salvo incorporated DNA and calcium chloride to activate DNase enzyme. Schreier modified DNase medium by adding toluidine blue ⁽³⁾. This modified medium achieved faster identification of Serratia marcescens and could differentiate Serratia from other members of the Enterobacteriaceae. DNase Test Agar Base without DNA can be used to detect DNase activity as well as mannitol fermentation by the addition of mannitol and a pH indicator dye i.e. bromothymol blue ⁽⁵⁾.

Casein enzymic hydrolysate or papaic digest of soyabean meal provides essential nutrients. The depolymerization of the DNA (DNase activity) may be detected by flooding the surface of the medium with 1 N HCl ⁽⁴⁾ and observing for clear zones around the colonies on the medium (with added DNA and mannitol and no bromothymol blue). In the absence of DNase activity, cloudy precipitate is formed due to reaction of HCl with nucleic acids. When bromothymol blue is used, yellow zones are formed.

Further confirmatory tests for the identification should be carried out.

Methodology

Suspend 40.0 grams of powder media in 1000 ml distilled water. Add 2 grams of DNA, 0.025 grams Bromothymol blue and 10 grams of mannitol. Heat, to boiling, 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 Petri plates.

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 Bromothymol blue : Blue coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.2% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH range 7.10-7.50

Cultural Response/ characteristics

DM 1741: Cultural characteristics observed with added 2 grams of DNA, 0.025 grams Bromothymol blue and 10 grams of mannitol after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	D-Nase Activity
Serratia marcescens ATCC 8100	50-100	luxuriant	positive reaction ,change in colour from green to yellow around the growth positive reaction ,change in colour from green to yellow
Staphylococcus aureus ATCC 25923	50-100	luxuriant	around the growth
Staphylococcus epidermidis ATCC 12228	50-100	luxuriant	negative reaction positive reaction ,change in colour from green to yellow
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	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° 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.
5. MacFaddin J. F., 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1. Williams & Wilkins, Baltimore, Md.

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