

TM 710 – DNASE TEST AGAR BASE (W/O. DNA AND TOLUIDINE BLUE)

INTENDED USE

With the addition of DNA, it is used for detection of deoxyribonuclease activity of bacteria and fungi.

PRODUCT SUMMARY AND EXPLANATION

DNase Test Agar Base is used for detecting deoxyribonuclease activity of bacteria and fungi and particularly for identification of pathogenic Staphylococci. With added toluidine blue, it is used in differentiation and identification of non-pigmented *Serratia* species isolated from clinical sources that might be improperly identified as *Enterobacter* and *Klebsiella* species. DNase activity was observed by Weckman and Catlin in Micrococci and found the correlation with coagulase activity as coagulase positive species were DNase positive. Di Salvo 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. 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.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	15.000
Soya peptone	5.000
Sodium chloride	5.000
Agar	15.000

PRINCIPLE

The medium consists of Tryptone and soya peptone which provides essential nutrients. The depolymerization of the DNA (DNase activity) may be detected by flooding the surface of the medium with 1 N HCl 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.

INSTRUCTION FOR USE

Dissolve 40.0 grams in 1000 ml purified/distilled water. Add 2 grams of DNA, 0.025 grams Bromothymol blue and 10 grams of mannitol.

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- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 12 to 15 psi pressure (118°C to 121°C) for 15 minutes. Cool to 45-50°C.
- Mix well and pour in sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: After addition of Bromothymol blue : Blue coloured, clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C)	: 7.3 ± 0.2





INTERPRETATION

Cultural characteristics observed with added 2 grams of DNA, 0.025 grams Bromothymol blue and 10 grams of mannitol after incubation.

Microorganism	АТСС	Inoculum (CFU/ml)	Growth	Recovery	DNase Activity	Incubation Temperature	Incubation Period
Serratia marcescens	8100	50-100	Luxuriant	>=70%	Positive reaction, change in colour from green to yellow around the growth	35-37°C	18-24 Hours
Staphylococcus. aureus	25923	50-100	Luxuriant	>=70%	Positive reaction, change in colour from green to yellow around the growth	35-37°C	18-24 Hours
Staphylococcus epidermidis	12228	50-100	Luxuriant	>=70%	Negative reaction	35-37°C	18-24 Hours
Streptococcus pyogenes	19615	50-100	Luxuriant	>=70%	Positive reaction, change in colour from green to yellow around the growth	35-37°C	18-24 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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- 5. Schreir, 1969, Am. J. Clin. Pathol., 51:711.
- 6. Streitfeld, Hoffman and Janklow, 1962, J. Bact., 84:77.
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NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. *For Lab Use Only

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