# DNase Test Agar Base w/o DNA

**M741**

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

## Directions

Suspend 40.0 grams 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.

## Principle And Interpretation

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 nonpigmented *Serratia* species isolated from clinical sources that might be improperly identified as *Enterobacter* and *Klebsiella* species. DNase activity was observed by Weckman and Catlin (1) in Micrococci and found the correlation with coagulase activity as coagulase positive species were DNase positive. 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.

## Quality Control

**Appearance**
Cream to yellow homogeneous free flowing powder

**Gelling**
Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
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**
7.10-7.50

**Cultural Response**

M741: 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 |
| *Staphylococcus aureus ATCC 25923* | 50-100 | luxuriant | positive reaction, change in colour from green to yellow around the growth |
| *Staphylococcus epidermidis ATCC 12228* | 50-100 | luxuriant | negative reaction |
| *Streptococcus pyogenes ATCC 19615* | 50-100 | luxuriant | positive reaction,change in colour from green to yellow around the growth |

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

## Reference

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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