DNase Test Agar

For detecting microbial DNase (deoxyribonuclease) by the method of JEFFRIES et al. (1957) and for identifying microorganisms, especially DNase-positive staphylococci.

This culture medium complies with the recommendations of the International Organization for Standardisation (ISO) (1977).

Mode of Action

Colonies producing DNase hydrolyse the deoxyribonucleic acid (DNA) content of this medium located in their immediate vicinity. If the medium is then flooded and acidified with 1 N HCl, the DNA precipitates out (turbidity) and clear zones appear around DNase-positive colonies. Some authors recommend instead flooding the medium with toluidine blue solution (STREITFIELD et al. 1962) or the use of DNase test agars containing toluidine blue (SCHREIER 1969) or methyl green (SMITH et al. 1969).

Staphylococci can also be differentiated by exploiting the fact that they metabolize mannitol to form acid, in this case mannitol and a pH indicator must be added to the culture medium.

Typical Composition (g/litre)

Tryptose 20.0; sodium chloride 5.0; deoxyribonucleic acid 2.0; agar-agar 15.0.

Preparation

Suspend 42 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.3 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Addition of mannitol: Prior to autoclaving the culture medium add 10 g mannitol/litre and, as an indicator, 0.025 g bromothymol blue/litre or 0.025 g phenol red/litre and mix thoroughly.

Experimental Procedure and Evaluation

Inoculate by streaking a pure culture of the organism to be tested onto the surface of the test agar. Several strains can be inoculated onto one plate (divide the plate into sectors or make parallel streaks).

Incubation: under optimal conditions (in the case of staphylococci, 24 hours at 35 °C aerobically).

When necessary first check the plates for mannitol fermentation, then carefully flood the surface of the plates with 1 N hydrochloric acid.

Appearance of Colonies	Microorganisms
Mannitol:	
Yellow, surrounded by a yellow zone	Mannitol-positive
Colourless or the same colour as the culture medium	Mannitol-negative
1 N HCl:	
Well defined, clearer zones in an otherwise turbid culture medium	DNase-positive
No clear zones	DNase-negative

Literature

International Organization for Standardization: Meat and meat products -Detection and enumeration of Staphylococcus aureus (Reference methods). **-Draft International Standard ISO/DIS 5551** (1977).

JEFFRIES, C.D., HOLTMANN, D.F., a. GUSE, D.G.: Rapid method for determining the activity of microorganisms on nucleic acid. **- J. Bact., 73**; 590-591 (1957).

SCHREIER, J.B.: Modification of Deoxyribonuclease Test Medium for rapid identification of Serratia marcescens. **- Amer. J. Clin. Pathol., 51**; 711-716 (1969).

SMITH, P.B., HANCOCK, G.A., a. RHODEN, D.L.: Improved Medium for Detecting Deoxyribonuclease-Producing Bacteria. **- Appl. Microbiol., 18**; 991-993 (1969).

STREITFIELD, M.M., HOFFMANN, E.M., a. JANKLOW, H.M.: Evaluation of extra-cellular deoxyribonuclease activity in Pseudomonas. **- J. Bact., 84**; 77-80 (1962).

Ordering Information

Product	Merck Cat. No.	Pack size
DNase Test Agar	1.10449.0500	500 g
Bromothymol blue indicator	1.03026.0005	5 g
D(-)Mannitol	1.05982.0500	500 g
Hydrochloric acid 1 mol/l	1.09057.1000	1 l
Phenol red indicator	1.07241.0005	5 g

DNase Test Agar

Quality control

Test strains	Growth	Clear zones
Staphylococcus aureus ATCC 25923	good / very good	+
Staphylococcus aureus ATCC 6538	good / very good	+
Staphylococcus epidermidis ATCC 12228	good / very good	-
Escherichia coli ATCC 25922	good / very good	-
Serratia marcescens ATCC 14756	good / very good	+
Bacillus cereus ATCC 11778	good / very good	+

Staphylococcus aureus ATCC 25923

Escherichia coli ATCC 25922