SIGMA-ALDRICH®
sigma-aldrich.com
3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

70136 Deoxyribonuclease Test Agar (DNase Test Agar)

A diagnostic medium for the detection of coagulase-positive DNase producing Staphylococci.

Composition:

Ingredients	Grams/Litre
Casein enzymatic hydrolysate	15.0
Papaic digest of soybean meal	5.0
Deoxyribonucleic acid	2.0
Sodium chloride	5.0
Agar	15.0
Final pH 7.3 +/- 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance:	Faintly yellow to brown colored, homogeneous, free flowing powder.
Gelling:	Firm
Color and Clarity:	Lightly yellow to brown colored, clear to slightly opalescent gel forms in petri plates.

Directions:
Suspend 42 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. After 18 hours incubation flood the plates with normal HCl and look for clearing around the colonies.

Bacteria are streaked on to the surface of the agar medium and incubated. After inoculation and 18-24 hours incubation the growth on the surface of the agar is flooded with 1N hydrochloric acid. Polymerised DNA precipitates in the presence of 1N HCl and makes the medium opaque. If the organisms produce DNase enzymes, in sufficient quantity to hydrolyse the DNA, then clear zones are seen around the colonies.

Principle and Interpretation:
Casein enzymatic hydrolysate is a source of nitrogen, vitamins, amino acids and other essential growth nutrients. Deoxyribonucleic acid (DNA) can be hydrolysed by microorganisms producing DNase. If the medium is then flooded with 1 N HCl not hydrolysed DNA precipitates (turbidity) and around DNase-positive colonies clear zones can be observed. Sodium chloride maintains the osmotic balance of the medium and Agar is the solidifying agent.

Instead of flooding the medium with 1N HCl it is also possible to use toluidine blue, crystal violet or methyl green as an indicator [4,5,6]. With the addition of indicator a faster identification of *Serratia marcescens* is achieved. Gram-negative DNase producing bacilli grow on this medium with indicator may accepted as *Serratia* species. May some strains of Staphylococci are inhibited by using indicator.
Staphylococci can also be differentiated by ability to ferment mannitol. This can be determined simultaneously by adding 10g/l mannitol and 0.025 g/l of phenol red (a pH indicator turns from red to yellow) to the culture medium.

Cultural characteristics after 24-48 hours at 35±2°C.

Organisms (ATCC)	Growth	DNase*
Serratia marcescens (8100)	+++	+
Staphylococcus aureus (25923)	+++	+
Staphylococcus epidermidis (12228)	+++	-
Streptococcus pyrogenes (19615)	+++	+

* positive reaction: change in color from blue to pink purple around the colonies when toluidine blue is used /clear zone surounding colonies when plates are flooded with 1N HCl

References:

1. C.D. Jeffries, D.F. Holtmann, D.G. Guse, Rapid method for determining the activity of microorganisms on nucleic acid, J. Bact., 73, 590-591 (1957)
2. B.G. Weckman, B.W. Catlin, J. Bact. 73, 747 (1957)
3. J.W. DiSalvo, Med. Tech. Bull., U.S. Armed Forces Med. J. 9,191 (1958)
4. M.M. Streitfield, E.M. Hoffmann, H.M. Janklow, Evaluation of extra-cellular deoxyribonuclease activity in Pseudomonas, J. Bact., 84, 77-80 (1962)
5. J.B. Schreier, Modification of Deoxyribonuclease Test Medium for rapid identification of *Serratia marcescens*, Amer. J. Clin. Pathol., 51, 711-716 (1969)
6. P.B. Smith, G.A. Hancock, D.L. Rhoden, Improved Medium for Detecting Deoxyribonuclease-Producing Bacteria, Appl. Microbiol., 18, 991-993 (1969)
7. International Organization for Standardization (ISO), Meat and meat products - Detection and enumeration of *Staphylococcus aureus* (Reference methods), Draft ISO/DIS 5551 (1977).

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.