DNase Agar (NCM0161)

Intended Use
DNase Agar is used for the differentiation of microorganisms on the basis of deoxyribonuclease activity and is not intended for use in the diagnosis of disease or other conditions in humans.

Description
In 1956, Weckman and Catlin showed a correlation between increased DNase activity of *Staphylococcus aureus* and positive coagulase activity. Their research suggested DNase activity could be used to identify potentially pathogenic staphylococci. DiSalvo confirmed their results by obtaining excellent correlation between coagulase and DNase activity of staphylococci isolated from clinical specimens. Jeffries, Holtman, and Guse incorporated DNA in agar to study DNase production by bacteria and fungi. Polymerized DNA precipitates in the presence of 1N HCl, creating an opaque medium. Organisms that degrade DNA produce a clear zone around an inoculum streak. Fusillo and Weiss studied calcium requirements of staphylococci for DNase production, and concluded additional calcium was unnecessary when a complete nutritive medium was used.

Typical Formulation

Enzymatic Digest of Casein	15.0 g/L
Enzymatic Digest of Animal Tissue	5.0 g/L
Sodium Chloride	5.0 g/L
Deoxyribonucleic Acid	2.0 g/L
Agar	15.0 g/L

Final pH: 7.3 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
Refer to SDS

Preparation
1. Suspend 42 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.

Test Procedure
1. Inoculate plates by spotting or streaking a heavy inoculum of test organism.
2. Use a spot approximately 5 mm in diameter or a 1 – 2 cm streak approximately 5 mm wide.
3. Incubate plates at 35 ± 2°C for 18 – 24 hours and up to 48 hours.
4. Flood plates with 1 N HCl.
5. Observe for clearing around the spot or streak. Record results.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is light amber, trace to slightly hazy.

Expected Cultural Response: Cultural response on DNase Test Agar at 33-38°C after 18 - 24 hours incubation.

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Microorganism	Approx. Inoculum (CFU)	Expected Results (DNase)
Serratia marcescens ATCC® 8100	$>10^6$	Positive
Staphylococcus aureus ATCC® 25923	$>10^6$	Positive
Staphylococcus epidermidis ATCC® 12228	$>10^6$	Negative
Streptococcus pyogenes ATCC® 19615	$>10^6$	Positive

The organisms listed are the minimum that should be used for quality control testing.

Results

A zone of clearing around the spot or streak indicates DNase activity.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Composition of the medium, degree of aeration, pH temperature, and incubation period are important factors influencing DNase activity in culturing and testing staphylococci.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Weckman, B. G., and B. W. Catlin. 1957. Deoxyribonuclease activity of micrococci from clinical sources. J. Bacteriol. 73:747-753.
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3. Jeffries, C. D., D. F. Holtman, and D. G. Guse. 1957. Rapid method of determining the activity of microorganisms on nucleic acid. J. Bacteriol. 73:590-591.
4. Fusillo, M. H., and D. L. Weiss. 1959. Qualitative estimation of staphylococcal deoxyribonuclease. J. Bacteriol. 78:520.
5. MacFaddin, J. D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 275-284. Williams & Wilkins, Baltimore, MD.

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