**Intended Use**
DNase Test Agar, DNase Test Agar with Methyl Green and DNase Test Agar with Toluidine Blue are differential media used for the detection of deoxyribonuclease activity to aid in the identification of bacteria isolated from clinical specimens.

**Summary and Explanation**
The DNase test is used to detect the degradation of deoxyribonucleic acid (DNA). The test is useful for differentiating *Serratia* from *Enterobacter*, *Staphylococcus aureus* from coagulase-negative staphylococci, and *Moraxella catarrhalis* from *Neisseria* species.

In 1957, Jeffries et al. described a rapid agar plate method for demonstrating DNase activity of microorganisms. This procedure utilized a semi-synthetic medium with nucleic acid solution incorporated in the medium. Enzymatic activity is detected by flooding the plate with 1 N hydrochloric acid (HCl). A clear zone surrounding growth indicates a positive reaction.

**Principles of the Procedure**
Peptones provide amino acids and other complex nitrogenous substances to support bacterial growth. Sodium chloride maintains osmotic equilibrium. DNA is the substrate for DNase activity. DNase is an extracellular enzyme that breaks the DNA down into subunits composed of nucleotides.

The depolymerization of the DNA may be detected by flooding the surface of the medium with 1 N HCl and observing for clear zones in the medium surrounding growth. In the absence of DNase activity, the reagent reacts with the intact nucleic acid, resulting in the formation of a cloudy precipitate.

The HCl reagent is not needed to detect DNase activity on DNase Agar with Methyl Green. Methyl green forms a complex with intact (polymerized) DNA to form the green color of the medium. DNase activity depolymerizes the DNA, breaking down the methyl green-DNA complex, which results in the formation of colorless zones around colonies of the test organism. A negative test is indicated by the absence of a colorless zone around the colonies.

The HCl reagent is not needed to detect DNase activity on DNase Agar with Toluidine Blue. Toluidine blue forms a complex with intact (polymerized) DNA. In the intact DNA complex, the toluidine blue has the normal blue color. DNase activity depolymerizes the DNA, breaking down the dye-DNA complex. In the presence of nucleotides produced from the DNase depolymerization, the dye takes on its metachromatic color, forming pink to red zones around bacterial growth. A negative test is indicated when the medium remains blue.

DNase Test Agars
DNase Test Agar • DNase Test Agar with Methyl Green
DNase Test Agar with Toluidine Blue
User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both Difco™ and BBL™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ DNase Test Agar
Dehydrated Appearance: Light beige, free-flowing, homogeneous.
Solution: 4.2% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent, may have a slight precipitate.
Prepared Appearance: Light to medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C: pH 7.3 ± 0.2

BBL™ DNase Test Agar with Toluidine Blue
Dehydrated Appearance: Fine, homogeneous, free of extraneous material.
Solution: 4.2% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance: Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 4.2% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Difco™ DNase Test Agar or DNase Test Agar with Methyl Green
Prepare the medium per label directions. Inoculate by streaking with a line of undiluted culture across the medium and incubate at 35 ± 2°C for up to 48 hours. For DNase Test Agar, flood the plates with 1N HCl and examine for clear zones around the streaks (positive reactions). For DNase Test Agar with Methyl Green, examine the streak plates for decolorized zones around the streaks (positive reactions).

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>RECOVERY</th>
<th>REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>25923</td>
<td>Good</td>
<td>+</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>13048</td>
<td>N/A</td>
<td>Good/–</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>33495</td>
<td>Good/–</td>
<td>Good/–</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>13880</td>
<td>Good/+</td>
<td>Good/–</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>12228</td>
<td>Good</td>
<td>–</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>25923</td>
<td>Good/+</td>
<td>N/A</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>19615</td>
<td>Good</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>12228</td>
<td>Good/+</td>
<td>N/A</td>
</tr>
</tbody>
</table>

BBL™ DNase Test Agar
Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 18-24 hours. For DNase Test Agar, flood the plates with 1N HCl and examine for deoxyribonuclease activity. For DNase Test Agar with Toluidine Blue, examine for deoxyribonuclease activity.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>RECOVERY/REACTION</th>
<th>DNASE TEST AGAR W/TOLUIDINE BLUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter aerogenes</td>
<td>13048</td>
<td>N/A</td>
<td>Good/–</td>
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<tr>
<td>Klebsiella pneumoniae</td>
<td>33495</td>
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</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>12228</td>
<td>Good/+</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Formulae

Difco™ DNase Test Agar
Approximate Formula* Per Liter
Tryptose .......................................................... 20.0 g
Deoxyribonucleic Acid ....................................... 2.0 g
Sodium Chloride .............................................. 5.0 g
Agar ............................................................ 15.0 g

BBL™ DNase Test Agar
Approximate Formula* Per Liter
Pancreatic Digest of Casein .................................. 15.0 g
Papain Digest of Soybean Meal .............................. 5.0 g
Deoxyribonucleic Acid ........................................ 2.0 g
Sodium Chloride .............................................. 5.0 g
Agar ............................................................ 15.0 g

Difco™ DNase Test Agar with Methyl Green
Approximate Formula* Per Liter
Pancreatic Digest of Casein .................................. 10.0 g
Proteose Peptone No. 3 ........................................ 10.0 g
Deoxyribonucleic Acid ........................................ 2.0 g
Sodium Chloride .............................................. 5.0 g
Agar ............................................................ 15.0 g
Methyl Green .................................................. 0.05 g

Directions for Preparation from Dehydrated Product

DNase Test Agar or DNase Test Agar with Methyl Green
1. Suspend 42 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

Difco™ & BBL™ Manual, 2nd Edition
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

**Procedure**

Inoculate by making a single streak line using inoculum from an agar slant or plate. One plate may be inoculated with up to eight isolates by spot inoculation (1/8 to 1/4 inch) or streak inoculation (a single 1- to 2-inch line).

Incubate at 35 ± 2°C for 24-48 hours. Plates should be incubated in an inverted position. Incubate tubes with loosened caps.

Following incubation, flood DNase Test Agar plates with 1N HCl reagent and observe for reaction. Reagent addition is not required with DNase Test Agar with Methyl Green or with DNase Test Agar with Toluidine Blue.

**Expected Results**

A clear area surrounding growth (band/spot inocula) on DNase Test Agar after the addition of 1N HCl indicates a positive reaction, DNase activity. A negative reaction is indicated by no clearing and a cloudy precipitate around colonies and throughout medium due to precipitated salts in the medium.

A positive reaction on DNase Test Agar with Methyl Green is a distinct clear zone surrounding growth in an otherwise green-colored medium. The color of the medium remains unchanged if the test is negative.

On DNase Test Agar with Toluidine Blue, DNase activity is indicated by pink to red zones surrounding growth. The color of the medium remains unchanged if the test is negative.
References

Availability
Difco™ DNase Test Agar

Cat. No. 263220 Dehydrated – 500 g

BBL™ DNase Test Agar

Cat. No. 211179 Dehydrated – 500 g

Europe
Cat. No. 255506 Prepared Plates – Pkg. of 20*

Mexico
Cat. No. 227450 Prepared Plates – Pkg. of 10*

Difco™ DNase Test Agar with Methyl Green

Cat. No. 222020 Dehydrated – 500 g

BBL™ DNase Test Agar with Methyl Green

United States and Canada
Cat. No. 297202 Prepared Plates – Pkg. of 20*

Mexico
Cat. No. 211789 Prepared Plates – Pkg. of 10*

*Store at 2-8°C.