# DNase TEST AGAR

- For in vitro use only - Catalogue No. PD60 & PD61
TD61 & TD62

Our DNase Test Agar is used to differentiate between microorganisms based on their ability to produce the enzyme deoxyribonuclease (DNase).

In 1956, Weckman and Catlin showed a positive correlation between DNase activity and coagulase activity and suggested that DNase activity could be used to identify potentially pathogenic staphylococci. The original formulation was devised by Jeffries et al. whom incorporated DNA into trypticase soy agar; this medium required the addition of acid to detect polymerized DNA. The addition of dyes to the medium proved beneficial as the acid-addition step could be precluded; the addition of methyl green was reported by Smith, Handcock and Rhoden, and the toluidine blue O modification was reported by Schreier

For the methyl green modification, the dye methyl green is able to bind to polymerized DNA to form a stable green complex at pH 7.3. The action of DNase depolymerizes the DNA structure releasing methyl green; when not combined at this pH methyl green becomes colorless resulting in colorless halos around DNase-positive colonies.

Toluidine Blue O (TBO) is a metachromatic dye that changes color when complexed to different substances. When TBO complexes with polymerized DNA (uninoculated medium), a royal blue color results; when DNA is hydrolyzed, TBO complexes with oligonucleotides or mononucleotides and results in a change in the dye structure and absorption spectrum yielding a bright pink color. DNase-positive colonies appear with rose-pink halos on a blue background. Lior and Patel recommended the use of DNase Agar with TBO when testing *Campylobacter* species. It should also be mentioned that TBO might be inhibitory to some gram-positive bacteria including some strains of *Staphylococcus aureus*.

Uses for DNase Test Agar:

1. To identify potential pathogenic staphylococci. And to differentiate *Staphylococcus aureus* subsp. *aureus* (+) from *S. epidermidis* (–)
2. To aid in the differentiation between *Klebsiella*, *Enterobacter*, and *Serratia* spp. (+) except *S. proteamaculans* subsp. p*rotemacuans* (V+) and *S. fonticola* (–) from *Klebsiella-Enterobacter* (–)
3. To differentiate *Plesiomonas shigelloides* (–) from *Aeromonas* spp. (–)
4. To differentiate *Campylobacter jejuni* (V), *C. coli* (V+), *C. coli* (V+), *C. lari* (+), and *Helicobacter pylori* (+) from other *Campylobacter* spp. (–)
5. To differentiate *Moraxella catarrhalis* (+) from other frequently isolated *Moraxella* spp. (–)

**Formula per Litre of Medium**

| | |
|---|---|
| Pancreatic Digest of Casein | 15.0 g |
| Papaic Digest of Soybean Meal | 5.0 g |
| Sodium Chloride | 5.0 g |
| Deoxyribonucleic Acid | 2.0 g |
| Agar | 15.0 g |

pH 7.3 ± 0.2

**Additional Ingredients per Litre of Medium**

<u>PD60 DNase Test Agar with Methyl Green</u>

| | |
|---|---|
| Methyl Green | 0.05 g |

<u>PD61 DNase Test Agar with Toluidine Blue O</u>

| | |
|---|---|
| Toluidine Blue O | 0.1 g |

**Recommended Procedure**

1. Allow medium to reach room temperature prior to inoculation.
2. The inoculum should be from an overnight pure culture grown on Heart Infusion Agar, Sheep Blood Agar, or in BHI Broth. Using a direct inoculum, streak the plate from rim to center in a straight line (1 to 1½ inches long). Up to 8 test organisms can be streaked onto a single plate. If inoculating a tube, streak the tube from the bottom up in a fish tail motion.

3. Incubate aerobically at 35°C.
4. Examine after 24 hours. If growth is poor reincubate medium for an additional 24 hours until good growth is observed.

**Interpretation of Results**

PD60 DNase Test Agar with Methyl Green

DNase Positive (+):
Clear zones around bacterial streak and colonies against a green background. Clear zones are best observed against a white background.

DNase Negative (–):
No color change, medium remains green

PD61 DNase Test Agar with Toluidine Blue O

DNase Positive (+):
Bright rose-pink zone around bacterial streak or colonies against a royal blue background.

DNase Negative (–):
No color change, medium remains blue

Additional tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Many bovine strains of Staphylococcus species are inhibited by methyl green and toludine blue O therefore these mediums should not be used for testing staphylococci of animal origin*
- *According to Lior, methyl green is toxic to some strains of Campylobacter necessitating the use of the TBO formulation*

**Quality Control**

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

| Organism | Expected Result | |
|---|---|---|
| *Serratia marcescens* ATCC 13880 | (+) | MG: growth with clear zones around growth<br>TBO: growth with rose pink halos |
| *Klebsiella pneumoniae* ATCC 33495 | (–) | MG: growth with no clearing<br>TBO: growth with no color change |

**Storage and Shelf Life**

Our DNase Test Agars should be stored away from direct light at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions these mediums have a shelf life of 8 weeks from the date of manufacture.

**Ordering Information**

| Cat# | Description | Format |
|---|---|---|
| PD60 | DNase Agar w Methyl Green<br>[Standard 15x100-mm plate] | 10/pkg |
| PD61 | DNase Agar w Toluidine Blue<br>[Standard 15x100-mm plate] | 10/pkg |
| TD61 | DNase Agar w Toluidine Blue 5-mL<br>[16x100-mm Kim Kap tube] | 10/pkg |
| TD62 | DNase Agar w Methyl Green 5-mL<br>[16x100-mm Kim Kap tube] | 10/pkg |

## References


1. Weckman BG, Catlin BW. Deoxyribonucleic activity of micrococci from clinical sources. J Bacteriol 1957; 73:747-53.
2. Jeffries CD, Holtman F, Guse DG. Rapid method for determining the activity of microorganisms on nucleic acids. J Bacteriol 1957; 73:590-1.
3. DiSalvo JW. Deoxyribosnuclease and coagulase activity of micrococci. Med Tach Bull US Armed Forces Med J 1958; 9:191-6.
4. Smith PB, Hancock GA, Rhoden DL. Improved medium for detecting deoxyribonuclease-producing bacteria. Appl Microbiol 1969; 18:991-3.
5. Schreier JB. Modification of deoxyribonuclease test medium for rapid identification of *Serratia marcescens*. Am J Clin Pathol 1969; 51:711-6.
6. Lior H. New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis*. J Clin Microbiol 1984; 20:636-40.
7. Lior H, Patel A. Improved toluidine blue-DNA agar for detection of DNA hydrolysis by campylobacters. J Clin Microbiol 1987; 25:2030-1.
8. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
9. MacFaddin, JF. Biochemical tests for the identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.



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