PRODUCT SPECIFICATION SHEET

Nitrate Agar (DM178)

Intended Use
Nitrate Agar (DM178) is recommended for detection of bacteria on the basis of nitrate reduction.

Product Summary and Explanation
Nitrate Agar is prepared in accordance with the formula published in Pure Culture Study of Bacteria of the Society of American Bacteriologist.[1] Microorganisms may be differentiated according to their metabolism of certain substrates. The ability to reduce nitrate is valuable for differentiating and identifying various types of bacteria especially *Enterobacteriaceae* family.[2] Non-fermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable of denitrification, which is a reduction of nitrate to nitrogen gas. The production of gas from nitrate is an important differential test for glucose-nonfermenting gram-negative bacilli. The end product of reduction depends upon the bacterial species.[3]

Principles of the Procedure
Nitrate Agar contains peptic digest of animal tissues and beef extract which provides carbon, nitrogen and other essential nutrients required for growth of microorganisms. The medium also contains potassium nitrate which acts as a substrate for determining nitrate reduction by bacteria. Certain bacteria convert nitrate to nitrite, ammonia or nitrogen gas. The presence of nitrites can be detected by the addition of 0.5 ml each of sulphanilic acid (IR004) and alpha-naphthylamine solution (IR005).

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	5.00
Beef extract	3.00
Potassium nitrate	1.00
Agar	12.00
Final pH: 6.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 21 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense in tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Allow to cool the tubes in slanted position.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in tubes as slants
Reaction of 2.1% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.2% Agar gel

Expected Cultural Response: Cultural characteristics observed after incubation at 35 - 37°C for 18 - 24 hours. Nitrate reduction observed on addition of 0.5ml of Sulphanilic Acid (IR004) and 0.5ml of α-Naphthylamine Solution (IR005).

Sr.	Organisms	Results to be achieved

No.		Inoculum (CFU)	Growth	Nitrate reduction
1.	*Acinetobacter calcoaceticus ATCC 23055*	50 - 100	good-luxuriant	negative reaction
2.	*Enterobacter aerogenes ATCC 13048*	50 - 100	good-luxuriant	Positive reaction, distinct redpink colour developed within 1-2 minutes
3.	*Escherichia coli ATCC 25922*	50 - 100	good-luxuriant	Positive reaction, distinct redpink colour developed within 1-2 minutes
4.	*Salmonella Typhimurium ATCC 14028*	50 - 100	good-luxuriant	Positive reaction, distinct redpink colour developed within 1-2 minutes

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

Test Procedure

1. Prior to inoculation of Nitrate Agar, the organism to be tested must have been previously isolated on some other suitable solid medium. The use of a pure culture is essential to correct performance of the test.
2. Using a sterile inoculating loop remove several similar isolated colonies from the agar medium and using a sterile needle, stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant.
3. Replace cap loosely and incubate at 35-37°C.
4. Examine the tubes after 18-24 and 42-48 hours for growth in the medium tube.
5. After 24-48 hours add reagents as described in "Expected Results."
6. Members of *Enterobacteriaceae* characteristically reduce nitrate to nitrite which reacts with sulfanilic acid and N, N-dimethyl-1-naphthylamine to produce the red colour. This reaction is known as Griess reaction. If an organism grows rapidly and reduces nitrate actively, the test should be performed after an early incubation period since the nitrite may be further reduced to nitrogen.
7. For the test: Add few drops of each reagent i.e. sulphanillic acid (IR004) and a-naphthylamine solution (IR005) into the tube containing culture to be tested. A distinct red or pink colour indicates nitrate reduction. A control (un-inoculated) tube should also be tested. If there is no pink colour formation, add a pinch of zinc dust to confirm the absence of nitrate in the medium.

Results

1. If growth is apparent after 24-48 hours of incubation, examine the tubes.
2. Development of a red color within 2 minutes denotes a positive test for nitrate. The development of red violet colour is due to the formation of a red diazonium dye i.e. p-sulfobenzene-azo-a-naphthylamine, indicates nitrate reduction to nitrite.
3. If no colour develops, it means that either nitrate is not reduced or further reduction to ammonia or nitrogen gas has taken place.
4. This can be verified by adding a a pinch of zinc dust to the tube. Zinc reduces nitrate to nitrite resulting in a red colour. The red colour indicates that nitrate is still present and was not reduced previously. An absence of red colour after the addition of zinc dust indicates that no nitrate is present and thus the nitrate was reduced further than nitrite. Therefore the nitrate reduction test is evidenced by either the presence of a catabolic end product or the absence of nitrate in the medium.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

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

Limitations of the Procedure

1. Nitrate reduction is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, biochemical and serological tests. Consult appropriate texts for detailed information and recommended procedures.
2. Allow at least 2 minutes for the color to develop before considering the nitrate test negative.
3. The nitrate test is very sensitive. An uninoculated nitrate control should be tested with reagents to determine whether the medium is nitrate-free and that the glassware and reagents have not been contaminated with nitrous oxide.
4. The addition of too much zinc dust may result in a false-negative reaction or just a fleeting color reaction.

Packaging
Product Name : Nitrate Agar
Product Code : DM178
Available Pack sizes : 500gm

References

1. Society of American Bacteriologist, Pure Culture Study of Bacteria, 1944, 12 : Leaflet 11: 8.
2. Ewing, 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th ed., Elsevier Science Pub. Co., Inc., N.Y.
3. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.

Further Information

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