remel

Anaerobic Nitrate Reagent B

INTENDED USE
Remel Anaerobic Nitrate Reagent B is recommended for use in qualitative procedures for nitrate reduction in anaerobic bacteria.

SUMMARY AND EXPLANATION
Anaerobes found in soil and sediments are involved in such processes as nitrate reduction. Several methods are available to detect nitrate reduction.[1] In 1977, Wideman et al. utilized a Nitrate Disk to determine nitrate reductase production.[2] She found 89% test agreement with the more conventional Indole Nitrate Medium. Developed to serve the dual purpose of detecting indole production and nitrate reduction, Indole Nitrate Medium is an aid in the identification of a wide variety of microorganisms.

PRINCIPLE
Certain bacteria convert nitrate to nitrite, ammonia, or gas. This process is denitrification. Presence of nitrite is determined by the addition of sulfanilic acid which reacts with the nitrite to form a diazonium salt. 5-amino-2-naphthalene sulfonic acid then couples with the salt resulting in a red dye complex. However, if the organism converted nitrite to ammonia or nitrogen gas, no nitrate remains to react with the sulfanilic acid; therefore, zinc dust must be added. Zinc reduces nitrate to nitrite, resulting in a red color. The red color indicates that nitrate is still present and was not previously reduced. An absence of red color after the addition of zinc dust indicates that no nitrate was reduced further than nitrite.[3]

REAGENTS (CLASSICAL FORMULA)*

5-Amino-2-Naphthalene Sulfonic Acid (CAS 119-79-9)	1.33	g
Glacial Acetic Acid (CAS 64-19-7)	200.0	ml
Demineralized Water (CAS 7732-18-5)	800.0	ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
DANGER! POISON, may be harmful or fatal if swallowed. **CORROSIVE**, may cause burns or irritation to skin, eyes and respiratory tract. Avoid breathing vapor and eye/skin contact. **COMBUSTIBLE,** keep away from heat and flame.

This product is For *In Vitro* Diagnostic Use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information.

STORAGE
This product is ready for use and no further preparation is necessary. Store product in its original container at 20-25°C until used. Protect product from light.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, TRANSPORT
Specimens should be collected and handled following recommended guidelines.[4]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Nitrate Disk (REF 21093) or Indole Nitrate Medium (REF 061182), (7) Anaerobic Nitrate Reagent A (REF 21201), (8) Pipette, (9) Test tube, (10) Sterile Forceps, (11) Petri dish, (12) Zinc dust.

PROCEDURE
Disk Method:
1. Make a fresh subculture of the organism to be tested on a nonselective anaerobic blood agar plate. Streak heavily in one area.
2. Place the Nitrate Disk in the area of heavy inoculum using sterile forceps.
3. Incubate anaerobically at 35-37°C for 24-48 hours.
4. Remove the disk from surface of plate and place in a clean petri dish before adding reagents.
5. Add 1 drop each of Anaerobic Nitrate Reagents A and B to the disk. If no color develops in a few minutes, drop a small amount of zinc dust onto the disk surface and observe up to 5 minutes for color to develop.

Broth Method:
1. Prior to use, Indole Nitrate Medium should be boiled 2 minutes and cooled without agitation.
2. Inoculate medium with a fresh subculture of the test organism.
3. Incubate tube anaerobically with cap loosened at 35-37°C for 24-48 hours.
4. After incubation aliquot a portion of broth to a clean test tube for testing.

5. Add 5 drops each of Anaerobic Nitrate Reagents A and B to the aliquot. If no color develops in a few minutes, drop a small amount of zinc dust into the broth and observe up to 5 minutes for color to develop.

INTERPRETATION

Positive Test - Red color development after addition of Anaerobic Nitrate Reagents A and B; no red color development within 5 minutes after addition of zinc dust

Negative Test - No color change after addition of Anaerobic Nitrate Reagents A and B; red color development within 5 minutes after addition of zinc dust

QUALITY CONTROL

All lot numbers of Anaerobic Nitrate Reagent B have been tested using the following quality control organisms and have been found to be acceptable. Testing of a positive and negative control should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Veillonella parvula ATCC® 10790	Anaerobic, 48h @ 35°C	Positive
Clostridium sporogenes ATCC® 3584	Anaerobic, 48h @ 35°C	Negative

BIBLIOGRAPHY

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PACKAGING

REF 21211, Anaerobic Nitrate Reagent B..... 25 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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CAS (Chemical Abstracts Service Registry No.)

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