14305 Motility Nitrate Medium

Selective medium for motile nitrate-utilizing microorganisms (*Ps. aeruginosa, Cl. perfringens*).

Composition*:

Ingredients	Grams/Litre
Meat extract	3.0
gelatine peptone (pancreatic)	5.0
Potassium nitrate	5.0
Disodium hydrogen phosphate	2.5
Galactose	5.0
Agar	3.0

Final pH 7.4 ± 0.2 at 25°C

* formula adjusted to suit performance

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:
Dissolve 23.5 g in 1 litre distilled water and add 5 ml glycerol (Cat. No. 49769). Dispense into tubes with screw-caps and sterilize by autoclaving at 121°C for 15 minutes. Cool quickly in cold running water and allow the tubed medium to solidify in an upright position.

Inoculate the tubes and do not forget a negative control without any bacteria.

Nitrate test:
A successful nitrate reduction test is dependent on performing the test under the correct conditions. That means the organisms needs the accurate growth media, the correct temperature and anaerobic conditions. Nitrate reaction occurs only under anaerobic conditions. To reach an anaerobic condition it is may recommendable to over-gassing with e.g. carbon dioxide and seal the tube with parafilm or to incubate in an anaerobic jar (Cat. No. 28029).

Incubate the tubes at 35°C for 24 to 48 h in an incubator.

Put a 5 drops of reagent A and 5 drops of reagent B into the tube containing culture to be tested. A distinct red or pink colour, which should develop within a few minutes, indicates nitrate reduction.
If the medium turns pink-red before the addition of Zn powder, the reaction is positive, and the test is completed.
If it is colorless after the addition of reagents A and B, add a small amount ("sharp knife point") of zinc powder to the medium. Allow it to stand at room temperature for 10-15 min.
If the medium remains colorless after the addition of Zn powder, the test result is positive.
If the medium turns pink after the addition of Zn powder, the result is negative.
The negative control should also be tested. There should be no pink colour formation after adding reagent A and B and if zinc powder is added the colour should change to pink.

Addition of too much zinc powder can result in false-negative reaction.

Preparation of Reagents:
Sulfanilic acid solution (Reagent A): Dissolve 8 g of sulfanilic acid (Cat. No. 86090) in 1 litre 5N acetic acid. Store Reagent A at room temperature for up to 3 months, in dark. Reagents may be stored in dark brown glass containers; bottles may be wrapped in aluminum foil to ensure darkness.

α-Naphthylamine solution (Reagent B): Dissolve 6 g of N,N-Dimethyl-1-naphthylamine (Cat. No. 40860) in 1 litre 5N acetic acid. Store Reagent B at 2 to 8°C for up to 3 months, in dark. Reagents may be stored in dark brown glass containers; bottles may be wrapped in aluminum foil to ensure darkness.

Principle and Interpretation:
Motility Nitrate Medium is formulated in accordance with FDA (1) and APHA (2). A slight modification is recommended by ISO committee (3).
Gelatine peptone, meat extract and galactose provide essential nutrients for growth. Potassium nitrate is the substrate for the nitrate reduction. The presence of less quantity of agar in the medium makes it semisolid which allows detection of motility.
The pure cultures obtained from Thioglycolate Broth with Resazurine (Cat. No. 90404) or TSC Agar (Cat. No. 93745) are stab-inoculated on Motility Nitrate Medium and incubated at 35°C for 24 to 48 hours.

Nitrate reduction is not a confirmatory test. Complete identification should include the morphology, gram reaction, biochemical and serological tests.

Cultural characteristics after 24-48 hours at 35°C.

Organisms (ATCC)	Growth	Motility	Nitrate reduction
Clostridium perfringens (12924)	+++	growth along the stabline	+
Clostridium absonum (27555)	+++	weakly motile	weak or absent

References:

1. Bacteriological Analytical Manual, Food and Drug Administation, 8th ed., AOAC International, USA (1995)
2. C. Vanderzant, D. Splittstoesser (Eds.), Compendium of Methods for Microbiological Examination of Foods, 3rd ed., APHA Washington D.C. (1992)
3. International Organisation for Sandardization (ISO), Draft ISO/DIS 7937 (1985)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.