



Alpha-Naphthylamine Solution

R009

It is used along with Sulphanilic acid (R015) to determine nitrate reduction by bacterial strains.

Composition**

Ingredients

Naphthylamine	5.0gm
Acetic acid ,5N	1000.0ml

**Formula adjusted, standardized to suit performance parameters

Directions

Inoculate growth from an 18 - 24 hours pure culture into Nitrate HiVeg Broth, (MV439). Incubate at 35°C for 12 to 24 hours. Very rarely prolonged incubation upto 5 days may be required. Add 0.5 ml alpha -naphthylamine along with 0.5 ml sulphanilic acid (R015).

Principle And Interpretation

The a-Naphthylamine solution and Sulphanilic acid is used to determine nitrate reduction by bacterial strains. The reduction of nitrates (NO_3) leads to the formation of nitrites (NO_2) and may progress to the liberation of nitrogen gas. The nitrate reductase producing organisms reduce nitrate to nitrite which reacts with sulphanilic acid to form a diazonium salt. This salt reacts with a-naphthylamine to form a red coloured, water soluble azo dye which results in the visualization of pink-red colour. A distinct red colour formation within 1-2 minutes indicates reduction of nitrate to nitrite.

Quality Control

Appearance

Light pink coloured, clear liquid with characteristic odour.

Clarity

Clear with no insoluble particles.

Cultural Response

Add 0.5 ml. of 0.8% Sulphanilic Acid (R015) and 0.5 ml. Alpha-Naphthylamine Solution (R009) into 18-24 hours old cultures in Nitrate Broth (M439).

Organism	Growth	Nitrate Reduction
<i>Acinetobacter calcoaceticus</i> ATCC 43498	Luxuriant	Negative (No colour change)
<i>Enterobacter aerogenes</i> ATCC 13048	Luxuriant	Positive (Development of distinct red colour)
<i>Escherichia coli</i> ATCC 25922	Luxuriant	Positive (Development of distinct red colour)
<i>Salmonella Typhimurium</i> ATCC 14028	Luxuriant	Positive (Development of distinct red colour)

Storage and Shelf Life

Store at 10-30°C in tightly closed container. Use before expiry period on the label.

Reference

- 1) MacFaddin J., 1980, Biochemical tests for identification of medical bacteria.



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