Nitrate Reagent Discs (Twin Pack)  DD042

Nitrate reagent discs are used for detection of nitrate reduction by microorganisms.

Directions
Grow test culture on suitable Agar medium plate containing nitrate substrate. Place Part A (disc) on suspected colony. Add a drop or two of Part B (Rehydrating fluid) on the disc.

Principle And Interpretation
The test involves detection of the enzyme nitrate reductase which causes the reduction of nitrate in the presence of a suitable electron donor to nitrite, which can be tested by an appropriate colorimetric reagent. Almost all Enterobacteriaceae reduce nitrate. Nitrate reagent discs when placed on suspected colony turn red-pink in case of nitrate reduction (positive reaction), when a drop or two of Part B (Rehydrating fluid) is added to the disc.

Reduction of nitrate (NO3) to nitrite (NO2) and subsequently to nitrogen gas (N2) usually takes place under anaerobic conditions, in which an organism derives its oxygen from nitrate (1). Most facultative anaerobes can reduce nitrate in the absence of oxygen. This anaerobic respiration is an oxidation process in which inorganic substances furnish oxygen to serve as an electron acceptor to provide energy (2). The end product possibilities of nitrate reduction are many depending upon the bacterial species. The more common end product via nitrite reduction is molecular nitrogen (2). Depending upon environmental conditions, these products are usually not further oxidized or assimilated into cellular metabolism, but are excreted into the surrounding medium.

Quality Control
Appearance
Part A : Filter paper discs of 6 mm diameter bearing letters 'Nr' in continuous printing style.
Part B : Light brown coloured solution, may have black suspended particles

Cultural response
The Nitrate reduction reaction was observed after an incubation at 35-37°C for 18-24 hours, for various bacteria with Nitrate Reagent discs (Part A), soaked with a drop of Part B, using Nitrate Broth (M439).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Nitrate Reduction</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>luxuriant</td>
<td>positive reaction : red or pink colour formation on addition of nitrate reagent discs.</td>
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<tr>
<td>ATCC 25922</td>
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<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>luxuriant</td>
<td>positive reaction : red or pink colour formation on addition of nitrate reagent discs.</td>
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<tr>
<td>ATCC 13048</td>
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<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>luxuriant</td>
<td>positive reaction : red or pink colour</td>
</tr>
<tr>
<td>ATCC 14028</td>
<td></td>
<td></td>
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</tbody>
</table>
**Acinetobacter calcoaceticus**  luxuriant discs.

**ATCC 43498**

**Storage and Shelf Life**
Store at 2 - 8°C. Use before expiry date on the label.

**Reference**