



### Determining the presence/absence of Nitrate or Nitrite Reducing Bacteria

Used in oil, gas and petrochemical industries, air industries, food industries, water and waste water and other industries

Denitrification is very important from an environmental point of view because the nitrogen in the atmosphere ( $N_2$ ) is the result of this process and is carried out by nitrate and nitrite reducing bacteria (NRB). Therefore, NRB bacteria, as an indicator group of bacteria, are capable of decomposing organic materials containing nitrogen. These bacteria reduce nitrate to nitrite and then nitrogen gas. Complete denitrification is called reduction until reaching gaseous nitrogen.

If NRB bacteria are detected in the soil, it indicates the efficient functioning of the nitrogen cycle in the soil. If a large population of NRB bacteria is detected in the water, it indicates the presence of significant amounts of nitrates in the water. It is possible that besides the high amount of organic matter, the amount of oxygen in these waters is low and anaerobic conditions dominate.

Nitrite is usually used as a corrosion inhibitor in cooling water and liquids exposed to metals. Some microorganisms that normally use atmospheric oxygen have the ability to use nitrite as a source of oxygen. In this process, nitrite is reduced to nitrogen gas and immediately the liquid containing it becomes corrosive. In fact, the presence of NRB bacteria removes the elements that prevent corrosion. On the other hand, NRB bacteria can reduce corrosion by suppressing the growth of sulfide-reducing bacteria, removing hydrogen sulfide, and consuming hydrogen.

MicrobCheck™ NRB test kit is designed as a 50 ml falcon containing culture medium and floating ball.

### Manufacturer's Recommendation

Avoid contact with the inner wall of the falcon. Perform the test under sterile conditions.

After opening the Falcon, turn the door upside down and place it on a clean surface with its bottom facing the ground.

### Test Method

#### Preparation

Collect at least 25 ml of sample.

Pour 19 ml of the sample into the falcon and close it.

On the falcon, note the date and name of the specimen.



### Incubation

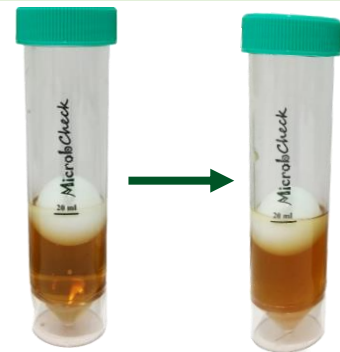
Incubate the falcon at room temperature (21-25°C) away from sunlight. Check the falcon containing the sample daily for 5 days. When the first reaction of clouding or bubbling is observed, check the result according to the result interpretation table. Daily inspection of falcons, especially in the first three days of incubation, is very important to determine the amount of contamination with NRB bacteria.

**Note that** if the examined samples are collected from environments with higher temperatures, incubate the falcons at the same temperature.

### Interpretation of results

This kit contains solution A and B as well as zinc powder, which is used to check the test result in the following way:

- 1- First, pour about three drops of solution A and three drops of solution B into a test tube, shake the tube until they mix well.
- 2- Take about 1 ml of the medium inside the falcon and add it to the tube containing solution A and B. Shake the tube until the solution is well homogenized.



- 3- Wait for about 2 minutes. The color change to red indicates the reduction of nitrate to nitrite. In this case, report the result as positive.

#### If the color change is not observed, proceed as follows:

- 4- Contact the sample with a very small amount of zinc powder, for example by dipping a toothpick or matchstick into the zinc. The result is possible in the following two ways:

- A) No color change, which means complete reduction of nitrate to gas. In this case, report the result as positive.
- b) Creating a red color, which means no reduction of nitrate. In this case, report the result as negative.

Note that adding large amounts of zinc powder will give a false negative test result.

### Reaction Pattern

The only reaction pattern in this test kit is foam formation (FO). In this pattern, the solution usually remains cloudy, but the main sign of the FO growth pattern is the presence of a large number of bubbles that cover more than 50% of the space around and under the ball. This pattern shows that denitrification has occurred completely and NRB bacteria are present in the sample. The estimation of the population of bacteria is calculated using the time lag of the formation of this foam.



### Estimation of Population and Aggression Level

The rate of changes in the culture medium inside the falcon is semi-quantitatively indicated by the number of NRB bacteria present in the sample. A positive result after one to two days indicates high NRB contamination (high aggression level), after two to three days indicates moderate contamination (moderately aggression level) and more than three days indicates low contamination (low aggression level).

**Note that** enteric bacteria are one of the largest groups of NRB bacteria, and *coliform* bacteria are also included in this group. *Coliforms* can perform denitrification in anaerobic and reducing conditions. Therefore, if a high amount of NRB bacteria is detected, it is necessary to investigate the presence of *coliform* bacteria.

If after two days, the test result is negative and no gas formation is observed, the population of bacteria is very low and it is considered as non-aggressive.

**Note That** the medium used in MicrobCheck™ NRB can support the growth of a variety of facultative aerobic bacteria and nitrate respiring bacteria. If there is another contamination in the sample under investigation, it can grow, which will be associated with the creation of cloud structures in the culture medium. This culture medium becomes more cloudy through time. If NRB bacteria are also present, they will be distinguished by gas production.

Aggression Level	Time Lag (day)	Population (cfu/ml)
Very Aggressive	1	1,000,000
Very Aggressive	2	200,000
Moderately Aggressive	3	50,000
Low aggressive	4	10,000

### Quality Control of the MicrobCheck™ NRB Test Kit

To confirm the quality and performance of the MicrobCheck™ NRB test kit, the specified strains can be cultured and the specified reaction patterns can be checked. After adding the bacterial dilution, wait until the suspension enters the culture medium and avoid shaking the falcon. Keep the falcon at room temperature and observe the reactions for 5 days.

Organism (ATCC)	Pattern
<i>Enterobacter aerogenes</i> (13048)	The formation of cloud structures with the production of gas or foam
<i>Pseudomonas aeruginosa</i> (27853)	Partial formation of cloud structures with gas or foam production
<i>Staphylococcus epidermidis</i> (12228)	Formation of cloud structures without producing gas or foam
<i>Acinetobacter calcoaceticus</i> (19606)	No formation of cloud structures with gas or foam production
<i>Escherichia coli</i> (25922)	The formation of cloud structures with the production of gas or foam



### Best Time to Use

The expiration date of the kits is 6 months and it is necessary to keep them in the refrigerator (4-8°C). It is recommended to avoid frequent temperature changes and storage in the freezer.

### Disposal

Test kits are completely contaminated after use and bacterial growth. As a result, they need to be autoclaved or burn in a furnace. If this is not possible, open the falcons under the laboratory hood and fill it with bleach liquid with a concentration of 5 to 10%. Let it sit overnight and then throw it away.

