



**To identify microbial corrosion caused by  
Thiosulfate-Reducing Bacteria**  
**Used in oil, gas and petrochemical industries, air  
industries, food industries, water and waste water, other  
industries with special formulations to check different  
samples including drinking water, waste water samples  
and food samples.**

The role of Sulfate-Reducing Bacteria (SRB) and Acid-Producing Bacteria (APB) in microbial corrosion of metal facilities has been well identified. It has been found that another group of sulfidogenic bacteria called Thiosulfate-Reducing Bacteria (TRB) are also involved in metal corrosion. Considering that it is not possible to identify TRB bacteria using other kits such as SRB, the MicrobCheck™ TRB kit was designed and manufactured.

Corrosion created in metals that are immersed in sea water leads to a lot of economic costs. The presence of Thiosulfate increases the possibility of biological corrosion in many oil field facilities and similar. The activity of Thiosulfate-Reducing Bacteria is also related to anaerobic corrosion formed in biofilms. This microbial population affects iron by producing hydrogen sulfide gas. In this way, Thiosulphate-Reducing Bacteria induce the formation of iron sulfide on metal surfaces by consuming hydrogen. The bad smell of hydrogen sulfide and metal corrosion is caused by the growth of Thiosulfate-Reducing Bacteria. Therefore, it is necessary to check the presence of these microorganisms regularly to control and prevent damage caused by their growth.

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

### Manufacturer's Recommendation

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

After opening the falcon, place the door upside down on a clean surface with its bottom facing the ground.

Do not shake or rotate the falcon after the sample is added. Let the ball float on the surface of the culture medium.

### Test Method

#### Sampling

**Samples soaked in oil:** Microorganisms cannot grow in the absence of water. In contaminated water/oil systems, microorganisms are found in the interface between water and oil and also in the water layer. Therefore, it is important to collect samples from these two parts. The sampling falcon must be filled with a fresh sample that has a minimum amount of oil on it. Excess oil on the surface of the sample can be removed with a dropper.



**Water sampling:** Collect at least 25 ml of the sample and pour 19 ml of the sample into the falcon and close it.

**Soil sampling:** Take 1 gr of the desired soil sample to the laboratory at a temperature of 4-15 °C. Dissolve the desired sample in 20 ml of sterile water and vortex. Wait for a while for the soil to settle. Then add water to the kit. Note that for tests that must be done on site and it is not possible to prepare a suspension, add 1 gr of soil directly to the kit and add up to 20 ml of sterile water.

Write down the date and name of the sample on the falcon.

**Note that** the presence of more than 20 ppm of hydrogen sulfide in the sample causes a false positive result. To remove hydrogen gas from the sample, pour 30 ml of the sample into a tube, close it and shake it for 10 seconds. Then let the tube stand still for 20 seconds. After that, you can use this sample for testing.

### Incubation

Keep the falcon at room temperature, i.e., 21-25 °C and away from sunlight.

Check the sample daily for 8 days. Note the date of the first observed reaction.

If the reaction is negative, keep the sample until the 14<sup>th</sup> day and check it daily.

### Presence / Absence

If there are TRB bacteria in the sample, after incubation, a ring of black slime will form around the ball, or black slime will form at the bottom of the falcon due to the growth of bacteria. In the absence of TRB bacteria, no black color can be seen in the solution.



### Estimation of Population and Aggression Level

If the test result is positive, you can estimate the bacteria population and their aggressive level according to the table below. A faster reaction occurs when the bacterial population is higher.

Aggression Level	Time Lag (day)	Population (cfu/ml)
Very Aggressive	1	6,800,000
Very Aggressive	2	700,000
Very Aggressive	3	100,000
Very Aggressive	4	18,000
Very Aggressive	5	5,000
Moderately Aggressive	6	1,200
Moderately Aggressive	7	500
Normal background	8	200



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

To confirm the quality and performance of the MicrobCheck™ TRB 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 kit at room temperature and check the reactions for 10 days.

Organism (ATCC)	Pattern
<i>Salmonella typhimurium</i> (14028)	Blackening of the culture medium
<i>Citrobacter freundii</i> (8090)	Blackening of the culture medium
<i>Enterobacter aerogenes</i> (13048)	Turbidity and color change to yellow
<i>Salmonella Typhimurium</i> (14028)	Turbidity and color change to yellow

### Best Time to Use

The expiration date of the kits is 6 months and it is necessary to store 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.

