



Determining the presence/absence of Sulfate-Reducing Bacteria in different water samples.

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

Sulfate-Reducing Bacteria (SRB) are a group of anaerobic bacteria that use hydrogen as the main source for many of their metabolic activities instead of oxygen. For this reason, sulfate-reducing bacteria are anaerobic and are inhibited in the presence of oxygen.

These bacteria have the common ability to reduce sulfate to hydrogen sulfide. The sulfide produced as a result of the growth of SRB bacteria reacts with metals (usually iron) and leads to the formation of black sulfides. As a result, it creates problems that start from the smell of rotten eggs and lead to the blackening of equipment, water and the formation of biofilm or slime and the beginning of microbial corrosion processes of metal facilities. One of the most important factors of steel corrosion in oil and gas industries is the corrosion caused by hydrogen sulfide gas. Many oil and gas facilities are made of carbon steel and are sensitive to corrosion by hydrogen sulfide gas.

It is difficult to identify SRB microorganisms due to their lack of growth in running water and their tendency to grow in depth and form biofilms (slime), and it is necessary to consider deep areas when sampling for testing. If the slimes are destroyed and the bacteria are left in the water, it is possible to identify SRB bacteria in running water. The MicrobCheck™ SRB test kit, with a modified and unique formulation, and based on NACE and API reference standards, increases the accuracy and ease of identification of SRB bacteria, and significantly reduces the time required for identification. In the SRB test kit, culture medium and conditions are used that provide selective conditions for anaerobic bacteria that are able to reduce sulfate. It also provides them with the required source of iron.

The MicrobCheck™ SRB 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, with the bottom facing the ground, on a clean surface.

Considering that sulfate-reducing bacteria do not grow directly in running water, but in the depths and in biofilms, make sure that sampling is done from the right place.

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



Test Method

Water Sampling

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.

Note that microorganisms cannot grow in the absence of water. In systems containing water and oil, organisms are found in the interphase and aqueous phase. Therefore, it is very important to sample these parts. Sampling vials should contain freshly collected samples from these phases and a thin layer of mineral oil on the surface of the samples. You can remove excess oil or oil from the surface of the sample with a dropper.

Soil Sampling

Transfer 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.

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.

Note that under the conditions of the SRB test, it may turn black in a very short time, for example, in less than 30 minutes. This is a sign of the presence of hydrogen sulfide in the sample, which quickly reacts with the iron in the SRB culture medium. It should be noted 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 the lid and shake for 10 seconds. Then let the tube stand still for 20 seconds. After that, you can use this sample for testing. In this condition, SRB bacteria are preserved by other bacteria in the slime and are not removed from the sample in the presence of oxygen.

Incubation

Incubate the falcon at room temperature 21-25°C and away from sunlight.

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

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

Presence / Absence

If SRB bacteria are present in the sample, after incubation, a black sediment will form at the bottom of the falcon. A ring of black slime around the ball or black slime is formed by bacterial growth at the bottom of the falcon. The results can be interpreted based on the type of bacterial growth in falcon.



Reaction Patterns

Different reaction patterns are observed in the case of growth of SRB bacteria in falcon:

Reaction number 1: Blackening Base (BB): The BB reaction is known by the formation of black sediment on the bottom of the falcon. At the beginning of sediment formation, it can be recognized by looking under the falcon. Then it gradually expands upwards. Blackening can also happen in the form of black dots around the walls of the falcon a little higher than the bottom cone of the falcon. In the BB reaction, the culture medium is clear and the slime ring around the ball is not visible. The BB reaction is a sign of the growth of anaerobic bacteria grown in deep areas such as *Desulfovibrio*.

Reaction number 2: Blackening around the ball (BT): In the BT reaction, a slime ring is seen around the ball. Slime is usually white, gray, beige or yellow in color and extends to the top of the ball. It should be noted that the formation of slime is not one of the features of this test kit and it is necessary to pay attention to its blackening. Blackening usually starts as black dots and slowly spreads inside the slime. The BT reaction is often a sign of the presence of slime-forming anaerobes, which includes SRB bacteria.

Reaction No. 3: Combination of Blackening in base and Ball (BA): In reaction 3, blackening is observed both in the base of the falcon and around the ball. In this situation, it was possible that the inner surface of the falcon was not blackened. In this case, the reaction is considered as type 3 or BA if the BB or BT states did not occur in the beginning. If one of the two reactions 1 or 2 occurred before 3, the focus should be on the earlier.

Two other types of reactions may also occur in the MicrobCheck™ SRB kit, which, although not a positive sign of the presence of SRB bacteria, are valuable because they can provide more information about the microbial activity in the sample.

Gas bubbles: sometimes observed on the first or second day of incubation in falcon of MicrobCheck™ SRB. By giving slow blows to the test falcon, these bubbles move from the lower part of the falcon and quickly reach the floating ball. It seems that this gas is methane produced by methane-producing bacteria grown in the highly reducing conditions of falcon's bottom.

Cloudy Gel-like (GC): in some samples, other anaerobic heterotrophic bacteria may also be present. These bacteria often grow faster than SRB bacteria. In this case, cloudy gel-like structures are formed, which are known as reaction number 4. GC structures are seen in the lower third of the test falcon. GC structures can expand and cloud the entire test liquid. It should be noted that these structures are stable and have clear boundaries. GC structures are not considered as a positive test for SRB. If the GC reaction is followed by BB or BT reactions, the presence of SRB bacteria can be considered positive.

Estimation of Population and Aggression Level

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



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™ SRB Test Kit

To confirm the quality and performance of the MicrobCheck™ SRB test kit, the specified strains can be cultured and 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>Enterobacter aerogenes</i> (13048)	Reaction 4
<i>Pseudomonas aeruginosa</i> (27853)	Reaction 4
<i>Proteus vulgaris</i> (13315)	Reaction 4
<i>Desulfovibrio desulfuricans</i> (DSM1924)	Reactions 1 and 3
<i>P. aeruginosa</i> (27853) + <i>D. desulfuricans</i> (DSM1924)	Reactions 2 and 3

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.

