
**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 in Vial test kit is designed as a 30 ml glass vial.

## Test Method

### Oil samples

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.

### Water and other samples

A special method for their sampling is not recommended.

### Incubation

Incubate the glass vial 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 28th day and check it daily.

## Presence / Absence

If the purpose of the test is to check the presence / absence of SRB bacteria in the sample, perform the test in the following way:

1- After sterilizing the metal cap on the glass vial with alcohol, remove it and sterilize the lower part again with alcohol.

2- Remove the syringe from the cover without touching the syringe head and connect it to the sterile needle in the cover. The tip of the syringe should not come into contact with anything non-sterile other than the sample.

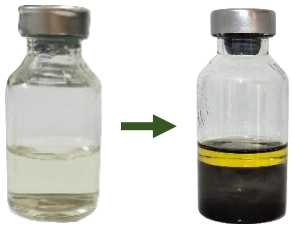
3- Remove the syringe needle from the cover and take 1 ml from the target sample.

4- Insert the needle of the syringe into the plastic cap of glass #1 that you have already cleaned with alcohol and slowly transfer the sample to the glass.

5- For better results, with a sterile syringe, add a few drops of oil (about 1 ml) to the glass to cover the surface of the sample.

6- Keep the samples in a closed box at room temperature or in an incubator at 21-25 °C.

7- The presence of SRB bacteria is determined by the darkening of the culture medium.



## Estimation of SRB Bacteria Population

## Interpretation of Results

If, in addition to the presence of SRB bacteria, there is a need to estimate the approximate population of these bacteria, perform the test by serial diluting in the following way:

1- With a marker, number the vials containing the culture medium from 1 to 5.

2- After sterilizing the metal cap part with alcohol, remove it and sterilize the lower part with alcohol again.

3- Remove the syringe from the cover without touching the syringe head and attach it to the sterile needle in the cover. The tip of the syringe should not come in contact with anything non-sterile other than the sample.

4- Remove the syringe needle from the cover and take 1 ml from the desired sample.

5- Insert the needle of the syringe into the plastic cap of vial number 1 and slowly transfer the sample to the vial and mix well.

6- Without removing the syringe from the cap, take 1 ml of the solution in the vial.

7- Remove the syringe from the cap and transfer the sample to vial number 2. Mix well.

8- Transfer the sample from vial 2 to vial no. 3. Mix well.

9- Transfer the sample from vial 3 to vial 4 using a 1 ml syringe and mix well.

10- Transfer the sample from vial 4 to vial 5 using a 1 ml syringe. Mix well and remove and discard 1 ml.

11- For better results with a sterile syringe, add a few drops of oil (about 1 ml) to the samples to cover the surface.
12- Keep all the vials in a closed box at room temperature or in an incubator at 25-30°C.

In this method, serial dilution method was used to check the approximate number of SRB bacteria. Note that the higher the number of SRB bacteria, the faster the culture medium changes color.

Periodically after 2, 5 and 15 days, check the test vials and record the positive results. If the vials do not change color, continue incubating for a maximum of 28 days. Check the number of vials that have changed color. The higher the number of vials that have changed color, the higher the number of SRB bacteria in the sample. Because even by diluting the sample, the number of bacteria is still so high that it has led to the darkening of the culture medium during the incubating period. In fact, if the last dilutions i.e., numbers 4 and 5 turn black, it means that the amount of SRB bacteria is very high because these dilutions have the lowest number of bacteria compared to the previous dilutions.

| The number of vials with positive results | The number of viable bacteria per ml of sample |
|---|---|
| **1** | 1 to 10 |
| **2** | 10 to 100 |
| **3** | 1,000 to 10,000 |
| **4** | 100,000 to 1 million |
| **5** | More than 10 million |

## Quality Control MicrobCheck™ SRB in Vial

To confirm the quality and performance of the MicrobCheck™ SRB in Vial test kit, the specified strains can be cultured and reaction patterns can be checked. Keep the vial at room temperature and check the reactions for 10 days.

| Organism (ATCC) | Pattern |
|---|---|
| *Enterobacter aerogenes* (13048) | Blackening of the culture medium |
| *Pseudomonas aeruginosa* (27853) | Blackening of the culture medium |
| *Proteus vulgaris* (13315) | Blackening of the culture medium |
| *Desulfovibrio desulfuricans* (DSM1924) | Blackening of the culture medium |
| *P. aeruginosa* (27853) + *D. desulfuricans* (DSM1924) | Blackening of the culture medium |

## 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

Glass vials 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 vials 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.