



## SRB VIALS

-For in vitro use only-

Catalogue No. ZAPI

Our SRB Vials are used for the detection and enumeration of sulfate-reducing bacteria (SRB).

The prevalence of sulfate-reducing bacteria is worldwide and they are of interest because of their connection with microbial-induced corrosion of metals. They have been found and associated with pipeline failures, oil souring, and have also been indicated in corrosion damage of water cooling systems. Symptoms of SRB-influenced corrosion include hydrogen sulfide odor (rotten-egg), blackening of waters, and black metal deposits. SRB are strict anaerobes that obtain their energy from the enzymatic reduction of sulfates. Hydrogen sulfide is a reactive end product that can react with certain metals, such as lead, iron or bismuth to form sulfides of these metals.

Our SRB vials are produced following recommendations put forth by the National Association of Corrosion Engineers (NACE) and the American Petroleum Institute (API). Our formulation contains yeast extract, which supply the organism with amino acids, vitamins and other complex nitrogenous components that are essential for growth. Some SRB strains can also utilize lactate therefore sodium lactate is added to the medium to foster the growth of these strains. Magnesium sulfate is present as a readily accessible source of sulfate for SRB. SRB reduce sulfates to form the colorless gas, hydrogen sulfide. Ferric ammonium sulfate reacts with hydrogen sulfide to produce ferrous sulfide, an insoluble black precipitate; this reaction is responsible for the observed blackening of the vials. The nails provide an additional source of iron and also act as reducing agents to keep the solution anaerobic. SRB are strict anaerobes therefore the medium has been pre-reduced to provide a suitable anaerobic environment for the bacteria.

In general, microorganisms are very sensitive to environmental changes therefore the salinity of the SRB vial should be proportional to the salinity of the test sample. Most common strains of SRB grow best

at temperatures ranging from 25°C to 35°C, but a few thermophilic strains can function efficiently at temperatures exceeding 60°C. The salinity and temperature of the sample should also be determined to maximize SRB recovery and growth rates.

### Components of Medium

Sodium Lactate, Yeast Extract, Ascorbic Acid, Potassium Phosphate, Ferric Ammonium Sulfate, Magnesium Sulfate, Sodium Chloride (variable)

pH 7.3 ± 0.2

### Recommended Procedure

1. Obtain six SRB Vials and label them 1 through 6. Prepare serial dilutions of the initial sample as indicated by the following procedure.
2. Fill a sterile, disposable 3-cc syringe with the test sample.
3. Expel any air from the syringe and any excess fluid until 1 cc of the test sample remains.
4. Inject the 1 cc of test sample into the first (#1) SRB vial and shake the vial to ensure homogeneity of the resulting solution.
5. Using a syringe, withdraw 1 cc from the first vial and inject it into the second (#2) vial. Shake the second vial to ensure homogeneity.
6. Repeat step 5 with the second vial and all subsequent vials to obtain serial dilutions of the initial sample.
7. If possible, maintain the incubation temperature within 5°C of the original sample temperature or if temperature was not determined incubate vials at 32°C.
8. Examine vials daily for the first week and intermittently for up to thirty days.

## Interpretation of Results

A positive reaction is a black, turbid solution throughout the vial. Formation of a black precipitate, ferrous sulfate, is indicative of sulfate reducing bacteria; turbidity alone is not indicative of SRB. SRB positive samples typically turn bottles black within 14 days although the rate of reaction is dependent on the SRB population and incubation temperature.

A negative reaction is indicated by either no growth in the liquid medium or a turbid solution with **no** black precipitate after 14 days. Occasionally late positives may develop after the 14 day period and vials may be kept for upto 30 days to check for late positives. Such lengthy incubation periods may be unnecessary for situations where it is known that late positives do not develop.

A rough estimate of the SRB population can be determined by analyzing the serial dilution data:

Number of Positive Vials	Actual Dilution of Sample	Growth Indicates (SRB / mL)	Reported as (SRB / mL)
1	1:10	1 to 9	10
2	1:100	10 to 99	100
3	1:1,000	100 to 999	1,000
4	1:10,000	1,000 to 9,999	10,000
5	1:100,000	10,000 to 99,999	100,000
6	1:1,000,000	100,000 to 999,999	≥1,000,000

- *Ensure that no gas is injected into the vials since an excess amount of hydrogen sulfide gas can produce false positives. Vials that turn black within two hours should not be considered positive since this is indicative of a sample with a high concentration of sulfide ion*
- *Some non-SRB organisms may also grow and produce hydrogen sulfide to give positive reactions*

- *As a general rule, culture media inherently underestimates bacterial population since only those organisms whose nutritional needs are met can grow and flourish*
- *Further dilutions can be made if desired, but 6 positive vials is indicative of a bacterial population above 1,000,000 per mL; a bacterial population this high already indicates a serious problem and further dilutions would be non-productive*
- *The serial dilution method provides a rough estimate of the bacterial population and is subject to statistical analysis. Therefore the more replicate samples done, the tighter the statistical distribution and the more precise the estimate*
- *If there is a gap between positive vials (for example one may find turbidity and blackening in vials 1, 2, 3 and 5, but not in vial 4. This can be explained in several ways:*
  - a) Accidental contamination of vial 5 occurred due to poor technique during inoculation.*
  - b) Only a few living bacterial cells may have been transferred into vial 4 from vial 3. These same cells may have been picked up during the 1 mL transfer from vial 4 to vial 5. The result would be growth in vial 5 but not in 4.*
  - c) The bacteria left in vial 4 failed to survive for some unknown reason, whereas the bacteria in vial 5 did.*

*In such cases, the interpretation is still based on the number of positive vials. So in this case the population would be read as 4 positive vials which is equal to 10,000 SRB/mL. Another alternative is to retest the sample to determine whether accidental contamination did occur.*
- *If samples are done in duplicate and show different estimates for the bacterial population in a water sample. Both results may be tabulated, or more often only the higher population range is reported.*

## Quality Control

The following organism can be used to determine the growth performance of the completed medium.

Organism	Expected Results
<i>Desulfovibrio desulfuricans</i> ATCC 29577	Turbidity (cloudiness) Black color change

## Storage and Shelf Life

Our SRB Vials should be stored away from direct light at 4 to 8°C. Under these conditions they have a shelf life of 26 weeks from the date of manufacture.

## Ordering Information

Cat#	Description	Format
ZAPI-0	SRB (API-38) 0 ppm [White Seals]	100/case
ZAPI-1	SRB (API-38) 1,000 ppm [Purple Seals]	100/case
ZAPI-5	SRB (API-38) 5,000 ppm [Gold Seals]	100/case
ZAPI-10	SRB (API-38) 10,000 ppm [Silver Seals]	100/case
ZAPI-25	SRB (API-38) 25,000 ppm [Blue Seals]	100/case
ZAPI-50	SRB (API-38) 50,000 ppm [Green Seals]	100/case
ZAPI-100	SRB (API-38) 100,000 ppm [Red Seals]	100/case

\* Custom salinities and cap colors are available upon request

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