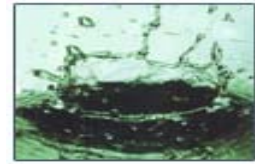




Strategic Diagnostics Inc.



# RapidChek® II SRB Detection System

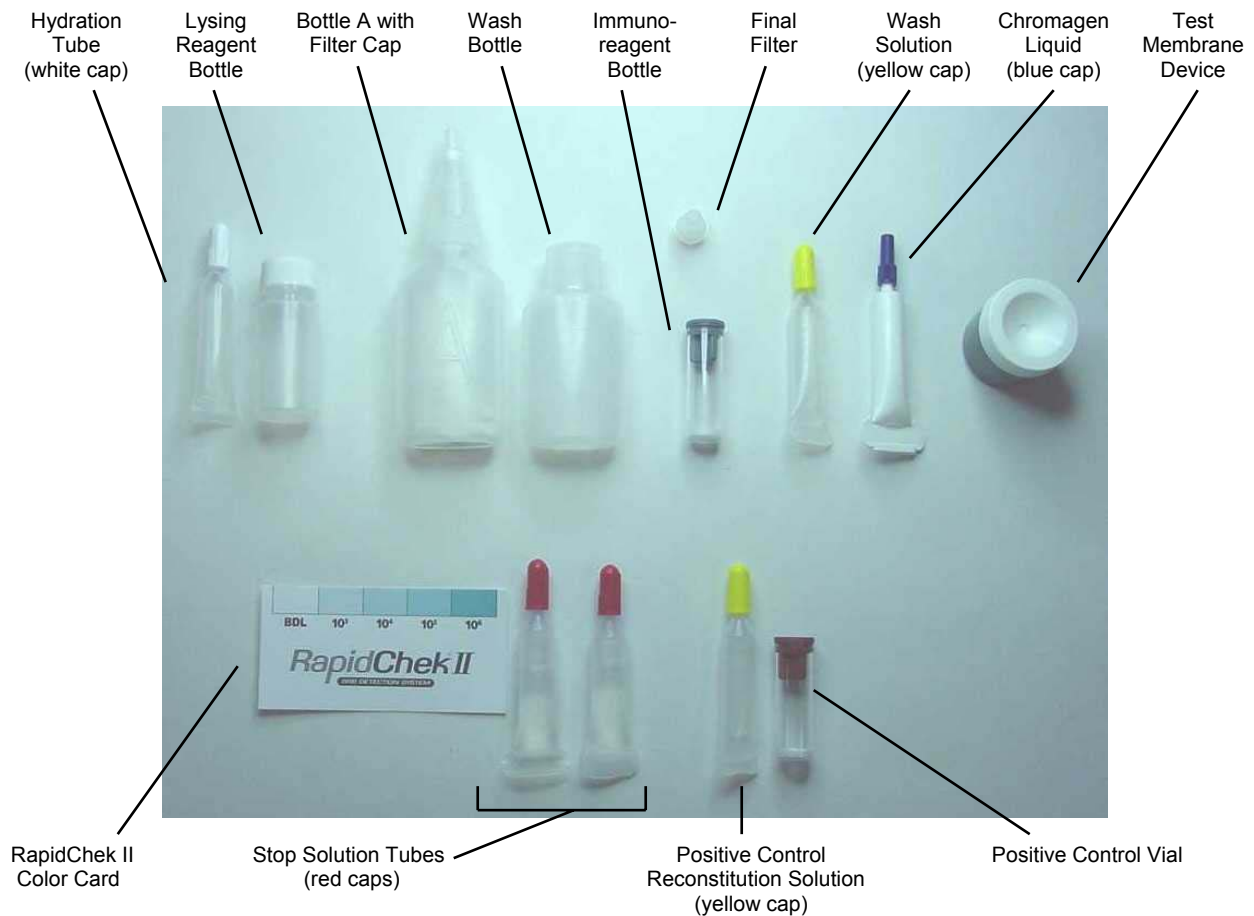
**Part Number SD50950 (10 Test)**  
**Part Number SD50951 (100 Test)**

## Introduction

The RapidChek® II SRB Detection System applies immunoassay technology to detect sulfate-reducing bacteria (SRB). This kit offers several advantages over traditional methods of SRB detection using cell culture techniques. These include immediate, accurate results, the ability to process solid and semi-solid samples, and the ability to detect all SRB, including SRB not able to grow in some standard media. Test results are not compromised by chemical or salinity interferences often found in field samples.

The RapidChek® II SRB test employs purified antibodies to detect the enzyme adenosine-5'-phosphosulfonate (APS) reductase which is common to all strains of SRB. This method for detecting and quantifying SRB is covered under U.S. Patent No. 4,999,286, owned by Strategic Diagnostic Inc.

The antibodies are attached to small particles that selectively capture the APS reductase enzyme. These particles and the captured enzyme are then isolated on a porous membrane, forming a reactive layer. This layer becomes blue in the presence of a chromagen, indicating the presence of the APS reductase enzyme. Darker shades of blue indicate a higher number of SRB. Each batch of reagents is calibrated to match known SRB quantities with distinct shades of this blue color.



## Precautions

Test kit fluids contain small quantities of sodium azide and other diagnostic chemicals and proteins. Exposure may cause skin irritation.

Avoid contact with eyes. Minimize skin exposure. Wash exposed areas with soap and water. In case of eye contact, flush eyes with water. If irritation persists, seek medical attention.

## Contents of the Kit

(see image on page 1)

- Hydration Tube (white cap)
- Lysing Reagent Bottle
- Sample Bottle A with Filter Cap
- Wash Bottle (contains 2 mL of wash buffer)
- Final Filter
- Immunoreagent Vial
- Wash Solution (yellow cap)
- Chromagen Liquid (blue cap)
- Test Membrane Device
- Stop Solution Tubes (red cap)
- Positive Control Reconstitution Tube (yellow cap)
- Positive Control Vial
- RapidChek®II Color Card

## Materials not Supplied

- Timer
- Vacuum Assist Package containing:
  - 250 mL Polypropylene flask with sidearm
  - Hand operated vacuum pump with gauge
  - #6 1/2 one-hole rubber stopper

While the RapidChek®II SRB test can be run on clean water samples without ancillary equipment, the vacuum assist package can make processing multiple clean water samples easier. In addition, turbid water samples and solid samples are difficult to process without the vacuum assist package. These materials are readily available from any scientific supply company.

## Assay Procedure

**NOTE:** Allow kit to reach room temperature prior to performing the test.

### Step 1 – Rehydrating the Chemical Lysing Reagent

Squeeze the contents of the white-capped tube into the lysing reagent bottle. This reactivates the freeze-dried lysing agent used to chemically dissolve the SRB cell walls (thus freeing the APS reductase enzyme). Set the bottle aside (for use in Step 3), and proceed to Step 2.

### Step 2 – Collecting the Sample

The Sample Bottle contains a small amount of a solid filtration medium. Tap the bottle on a hard surface to ensure that the filtration medium falls into the bottle before removing the cap. Remove the filter cap, and collect 10 mL of the liquid sample to be tested.

### Filtering and Washing the Sample (Without Vacuum Assist)

Reattach the filter cap tightly, turn the sample bottle upside down, and shake to suspend all filtration medium. Keep inverted *and wait 15 seconds*, allowing suspended solids to begin to settle on the filter plug in the cap. Then squeeze the sample bottle, expelling the sample liquid to waste. Remove all liquid. SRB originally present in the sample are now trapped in the filtration medium matrix.

*Caution:* While squeezing liquid from the sample bottle to waste, try not to release hand pressure on the bottle until all liquid is expelled. Releasing hand pressure will cause air to rush back into the bottle, upsetting the filter cake. This may result in a loss of captured bacteria.

After expelling the sample liquid, remove the filter cap containing the filtration material (and bacteria), and firmly attach it to the uncapped wash bottle. The wash bottle contains 2 mL of wash buffer.

Squeeze the wash bottle, expelling the wash liquid to waste through the filtration material. The SRB remain in the filter cap, but chemical impurities are washed away, removing potential interferences.

### Filtering and Washing Sample (Using Vacuum Assist)

**NOTE:** Sample washing is simplified when you use vacuum assist.

Once you have collected a 10 mL sample, reconnect the filter cap, shake, and invert the bottle into the single-hole stopper in the vacuum flask. Wait 15 seconds, then apply vacuum.

After expelling all sample liquid from the bottle, remove the sample bottle, leaving the filter cap inserted into the vacuum flask. Remove the cap from the wash bottle and pour wash into the filter cap. Apply vacuum again, if needed, to pull the wash through the filter cake to waste. Use of a vacuum also pulls all sample and wash liquid out of the filter cake, making subsequent processing steps easier.

### Step 3 – Chemical Cell Lysing

Remove the filter cap containing the filter cake from the wash bottle (or vacuum flask) and attach it to the lysing reagent bottle (that was set aside in Step 1). If you are using a hand vacuum pump, the filter cake will be tightly packed into the filter cap, and it will be free of

residual liquid. Flick the side of the lysing bottle/filter cap firmly with your index finger. If the filter cake does not become dislodged, turn the bottle 180° and flick it firmly again. After the filter cake is dislodged, strike the base on a flat surface to make the filter cake fall into the lysing fluid. Do not be timid!

If any of the cake remains in the filter cap, tip the bottle upside down and allow the fluid to run into the filter cap. When the fluid will go all the way to the plug in the cap, all the filter cake has been transferred.

Mix well, but avoid foaming. *Allow two minutes incubation.* The lysing reagent disrupts the SRB cell membranes, releasing the APS reductase enzyme into solution during incubation.

During the incubation, remove the filter cap from the lysing bottle and discard. Then snap the small final filter into the lysing bottle. Push hard until it “snaps” into place.

#### Step 4 – Antibody Enzyme Reaction

Remove the rubber cap from the glass immunoreagent vial. After lysing incubation ends (two minutes), squeeze the liquid from the lysing bottle into the immunoreagent vial. You should get between 10 and 20 drops of fluid. Stop squeezing the lysing bottle when all the liquid is expelled and foam appears at the filter tip. Mix the liquid in the immunoreagent vial gently and *let it incubate for two minutes.* During this incubation period, the antibody-coated particles bind with the APS reductase in solution.

Test results will not be affected if the steps are allowed to last up to four minutes.

#### Step 5 – Concentrating the Particles

Pour the contents of the immunoreagent vial onto the test membrane device. *Wait until the liquid is absorbed through the membrane,* and then squeeze the contents of the wash solution tube (yellow cap) onto the membrane. This washes the particles to ensure accurate results. It may take two of three minutes for the test liquid and wash liquid to go through the membrane.

*Wait until the wash solution is absorbed through the membrane,* and then squeeze the contents of the chromagen liquid (from the blue-capped tube) onto the membrane. Start timing the color development incubation immediately after squeezing chromagen on the device.

#### Step 6 – Interpreting Results

The speed of the enzyme color reaction for this step is affected by the ambient temperature. (It is 10 minutes at room temperature.) Allow the proper time to elapse as indicated on Chart 1, then match membrane color with the color card.

**Chart 1**

Temperature		Color Development Time (Minutes of Incubation)
°F	°C	
60-69	16-20	15
70-79	21-26	10
80-89	27-32	8
90-100	33-38	6

Do not run the test below 60°F (16°C) or above 100°F (38°C) ambient temperature.

Failure to use this ambient temperature guideline could lead to misinterpretation of test results. (This caution refers to the ambient temperature of the testing location and/or kit liquids. It does not refer to the sample liquid temperature, which does not affect RapidChek II results.)

At the proper time, read the color development by placing the RapidChek II color card next to the top of the funnel device. Match the color of the membrane to the color card to determine the SRB concentration (expressed in cells per milliliter). Bending the card to the place the appropriate color closer to the membrane can prove helpful.

**NOTE:** The color will continue to darken unless the enzyme reaction that creates the color is halted. The color of the membrane can be permanently fixed, if desired, using the stop solution (red capped tube found in the Accessory Kit). To preserve the membrane for future examination, end the color reaction by squeezing ten drops (0.4 mL) of the stop solution onto the membrane. This prevents continued color development to an inaccurate (darker) shade of blue.

Even samples containing no SRB will begin to develop blue color about five minutes after the appropriate color incubation time has elapsed; so reading or stopping color development at the proper time is very important.

#### Special Sample Processing Procedures

**NOTE:** Use of a vacuum pump is *essential* for processing dirty waters and solid samples unless the Alternative Solid Testing Procedure is followed.

#### Dirty Water Samples

If a water sample contains large concentrations of solids making filtration difficult, sample dilution may make processing easier. Add 5 mL of sample liquid to the sample bottle, and then fill to the 10 mL line with a clean (distilled or tap) water. This creates a 50% dilution. Shake and process as usual. If the sample will still not flow through the filter cap, stop and unscrew the filter. Dilute the sample further with additional clean water. Then try again. Diluting the sample with 15 mL instead of 5 mL will not appreciably change results since results are reported on a logarithmic scale.

Once sample fluid has been removed, complete remaining steps as with a normal sample.

### Solid Samples

The following procedure should be used in the processing of solids (biofilms, sludge, rust, mud, etc.): Collect a small amount (approx. 1 mL) of sample, and transfer it to the sample bottle. Dilute the solid with 10 mL of clean (distilled or tap) water and shake. Attach the filter to the sample bottle, invert, wait 15 seconds, and apply vacuum to expel the liquid. If the filter clogs, remove some solid material and/or dilute further; *or* you can use the alternative procedure that follows.

### Alternative Solids Testing Procedure

If you are testing a waxy sample or biofilm scraping that is impractical to wash, then filtration and washing (Step 2) can be skipped entirely.

Simply add the solid material (approx. 1/10 mL) to the activated chemical lysing agent. **NOTE:** Be sure to let the lysing reagent go into solution in the hydration fluid before adding the solid sample into the bottle.) After the two-minute incubation, complete remaining steps as with a normal sample.

If the sample is contaminated with a high level of SRB, then very little solid sample material will be required to generate a strong color response. If too much solid is added to the hydrated chemical lysing solution, solids carry-over onto the membrane can be a problem.

*Caution:* When testing solid samples, you must guard against the solid material absorbing the lysing solution. If it appears that the solids in the lysing bottle are absorbing all of the lysing reagent, it is advisable to activate another bottle of lysing reagent from an unused kit and add this extra reagent to the solid material in the bottle. This doubles the amount of lysing reagent, but ultimately only about half of this fluid will be expelled from the lysing bottle into the immunoreagent tube because some of was absorbed into the solids. It is important that at least 10 drops of the liquid are transferred into the immunoreagent vial.

### 100 mL Concentration Step (For enhancing detection limit to $10^2$ )

The standard detection limit of the RapidChek II SRB kit is  $10^3$  SRB cells per mL of water sample. This is calibrated with microscopically counted cells of the SRB strain *Desulfovibrio desulfuricans*. Many users want to see a lower concentration of SRB. The easiest way to enhance the detection limit of the kit is to collect additional cells from a larger water sample. To perform this concentration step, it is necessary to use a vacuum source and flask.

After processing the first 10 mL of sample, cut the bottom of Sample Bottle A while it is still inverted, and

process an additional 90 mL of sample water through the filter cap using the sample bottle as a funnel.

Approximately 10 times more SRB cells are captured with this procedure.

After processing 100 mL of water, follow the standard procedure for the rest of the test. Results will be enhanced by one log. Filtration will become slower as the 100 mL volume is approached. If the filter plugs after 80 or 90 mL have been processed, the enhancement is still valid.

This enhancement lowers the kit detection limit to  $10^2$  and will enhance the kit results by one order of magnitude across the entire test kit range. For example, if a 100 mL sample is processed and a  $10^3$  color response is obtained, the original sample actually contained  $10^2$  cells per mL.

The water used for enhancement must be relatively clean, or it will plug the prefilter; therefore, dirty water samples cannot be enhanced.

### Alternative Enhancement Procedure (For enhancing detection limit to $10^1$ )

Water samples with minimal amounts of particulate can be concentrated using the alternative method for trapping and lysing. This procedure allows a relatively large sample size to be filtered. The detection limit is lowered to  $10^1$  SRB per mL for a 1-liter sample.

#### Materials Required

1. Apparatus for pumping the water through the filter, such as a pump or vacuum system; or appropriate tubing and connectors to attach the filter in line.
2. Sterivex-HV filter, without bell, 0.45  $\mu$ m Durapore membrane, sterile, Millipore number SVHV01015 or equivalent.
3. Two (2 or 3 mL) syringes, without needles. Standard syringes will attach to the filter using Luer-Lok connections.

#### Procedure

1. Pump the desired volume of water through the Sterivex HV filter, and note the volume for accurate calculation of SRB per mL.
2. Displace as much water as possible from the filter by using a syringe to push air through the filter unit. Draw wash solution into the syringe. Attach the syringe to the Sterivex filter and push wash into the filter. Then drive wash fluid to waste by pushing air into the filter.
3. Draw the reconstituted lysing reagent into the syringe barrel. The volume should be about 0.75 mL.
4. Attach the Sterivex-HV filter unit to the syringe using the Luer-Lok end of the filter unit.
5. Carefully push the syringe plunger to deliver the chemical lysing fluid into the filter. Saturate the entire surface of the filter membrane by rotating the unit, being careful not to lose fluid from the open end of the filter.

Once the entire membrane is saturated with the lysing fluid, incubate two minutes.

6. Finally, expel the lysing fluid from the filter unit into the immunoreagent vial by pushing air into the filter with the syringe. Incubate two minutes, and complete remaining steps as with a normal sample.

**Calculation of the SRB per mL**

The detection limit of the kit can be enhanced by up to three orders of magnitude, depending on the amount of sample passed through the filter. For example, if a 100 mL sample is processed, the kit sensitivity will be one order of magnitude greater than if a 10 mL sample had been processed; and if a 1-liter sample is processed, the kit sensitivity will be two orders of magnitude greater than if a 10 mL sample had been processed. Therefore, if a 1-liter sample is processed and a 10<sup>3</sup> response is obtained, the original 10 mL sample actually contained 10<sup>1</sup> cells per mL.

**Additional Information**

**Shelf Life**

Each kit lot has an assigned expiration date based on ideal storage conditions (refrigerated, 38°F or 4°C). Shelf life decreases as the temperature increases. It is not recommended that the kits be stored above 100°F (38°C) as kit performance could be adversely effected.

**Using the Positive Control**

A positive control is shipped as part of the Accessory Kit that is included with each RapidChek®II SRB 10 or 100 test. The positive control should be used to verify the effectiveness of the kits.

To run the positive control, follow these easy steps:

1. Add the contents of the positive control reconstitution tube (yellow cap) to the positive control vial. Both tubes are found in the Accessory Kit. Mix gently to dissolve completely.
2. Pour the rehydrated positive control into a kit immunoreagent vial. Allow two-minute incubation as in the standard test.
3. Complete the remainder of the test according to standard procedures.

This positive control should yield a minimum of 10<sup>5</sup> color response.

**Ordering Information**

Description	Part Number
RapidChek II 10 Test	SD50950
RapidChek II 100 Test	SD50951
Accessory Pack <i>(positive control, stop and wash)</i>	SD50751

**Technical Assistance**

To Place an Order or Receive Technical Assistance, please call, fax, or email Strategic Diagnostics Inc.:

Call toll-free **800-544-8881**

Or 302-456-6789 Phone  
 302-456-6782 Fax  
 techservice@sdx.com

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P/N SD50158 Version 2.0, March 30, 2005 JMA