

PIPELINE PIT, FILM, & MIC BACTERIA KIT

Pit, Film, & MIC - Microbiology Survey

USER INSTRUCTIONS

SPECIAL NOTE: Before using this Pit, Film, & MIC Microbiology Survey kit, READ ALL INSTRUCTIONS. This kit only contains enough materials for one (1) test.

MICROBIOLOGY AND GENERAL CHEMISTRY

This kit will test for the following parameters:

- APBs - Acid Producing Bacteria (Red Cap)
- SRBs - Sulfate Reducing Bacteria (Green Cap)
- pH of Pit, Film, and MIC Areas

MICROBIOLOGY AND GENERAL CHEMISTRY TESTING

The testing is performed by the bottles, syringes, and sample template contained in this kit. The following instructions provide the procedures for the testing of pit, film, slime, undercoating, and MIC areas.

CAUTION: The sampling for the Microbiology Survey is to be completed first. **EXTREME** care must be used to keep the area to be sampled from being contaminated by touching, scraping the area with non-sterile tools, etc.

INSTRUCTIONS

MICROBIOLOGY SURVEY

- Step 1. If the area to be sampled is a pit (inside or outside the pipe or vessel), film on the surface, slime, sludge, or MIC area, proceed to Step 2. If the area to be sampled is an area under a bubble in the pipe coating, proceed to Step 7.
- STEP 2.** Open one end of the sealed plastic bag containing the sample template; set it aside until Step 3. Set the "ADS" tube (orange cap) upright in the bacteria box. Remove the cap, making sure to keep the cap clean. Now remove a sterile cotton tipped swab from the sealed paper bag. Make sure **NOT** to touch the cotton tip with your hands/fingers. Immerse only the cotton tip of the swab in the liquid in the open "ADS" tube. Take care not to spill any "ADS" liquid.
- Step 3. Holding the wetted cotton swab in one hand, use your other hand to remove the sample template from the plastic bag and place it over the area to be sampled. The sample template should be centered over the sample area. Holding the sample template in place, swab the area within the template cutout with the wetted tip of the swab. Start by rubbing the swab up and down within the template, starting at one side and proceeding across the template. Next swab the template area again, this time from left to right. **DO NOT** touch the cotton with your hands/fingers. Also, **DO NOT** let the cotton tip touch anywhere other than the area inside the sample template cutout.
- Step 4. With the area sampled, discard the sample template. In one hand pick up the open "ADS" tube, and hold it tightly. Now insert the cotton end of the swab into the "ADS" tube, approximately one inch. Snap the wooden stem of the swab, allowing the cotton end to fall into the "ADS" liquid.
- Step 5. Replace the orange cap onto the tube and tighten. Make sure the cap is secure. With the cap securely on the tube, shake the tube vigorously to mix the sample and the "ADS" liquid in the tube. The tube should be shaken for 30 to 45 seconds.
- Step 6. Set the mixed "ADS" tube upright in the bacteria box and allow the tube to sit undisturbed for 1 to 2 minutes. Now proceed with the pH test - Step 14.

- STEP 7. TO SAMPLE THE AREA UNDER A BUBBLE IN THE PIPE COATING:** The top of the bubble is to be cut with a sharp tool, such as a knife or flat blade screwdriver. Before slicing the bubble, the tool is to be sterilized. Clean the tool's blade of any dirt, debris, etc. Then remove one of the alcohol swabs and wipe the blade.
- Step 8. Gently cut a slice across the top of the bubble. Try not to apply too much pressure, so that the fluid/materials under the bubble are not pushed out.
- Step 9. Set the "ADS" tube (orange cap) upright in the bacteria box. Remove the cap, making sure to keep the cap clean. Now remove a sterile cotton tipped swab from the sealed paper bag. Make sure **NOT** to touch the cotton tip with your hands/fingers. Immerse only the cotton tip of the swab in the liquid in the open "ADS" tube. Take care not to spill any "ADS" liquid.
- Step 10. Insert the moistened sterile cotton tipped swab into the cut in the bubble slowly, allowing the cotton to absorb any fluid/material. Once the fluid/material is absorbed into the cotton, use the swab to clean the area under the bubble.
- Step 11. In one hand pick up the open "ADS" tube, and hold it tightly. Now insert the cotton end of the swab into the "ADS" tube, approximately one inch. Snap the wooden stem of the swab, allowing the cotton end to fall into the "ADS" liquid.
- Step 12. Replace the orange cap onto the tube and tighten. Make sure the cap is secure. With the cap securely on the tube, shake the tube vigorously to mix the sample and the "ADS" liquid in the tube. The tube should be shaken for 30 to 45 seconds.
- Step 13. Set the mixed "ADS" tube upright in the bacteria box and allow the tube to sit undisturbed for 1 to 2 minutes. Now proceed with the pH test - Step 14.

pH Testing

- Step 14. Remove the second sterile cotton swab from the sealed paper bag. Swab the same area that was previously swabbed for the Microbiology sampling. Thoroughly cover the cotton tip with the material to be sampled.
- Step 15. With the area thoroughly swabbed and the cotton portion of the swab covered/saturated, break the swab about an inch below the cotton end and place the cotton end in the DI water tube (blue cap). Replace the blue cap and tighten. Make sure the cap is secure. With the cap securely on the tube, shake the tube vigorously to mix the sample and the DI water in the tube. The tube should be shaken for 30 to 45 seconds.
- Step 16. Place tube upright in the box and allow the tube to sit for 1 to 2 minutes.
- Step 17. **pH Test.** Remove the cap from the blue tube and set the cap aside. Remove the pH strip from the plastic bag and fold the strip in half. Place the folded edge only of the pH strip into the liquid, making sure to wet only the tip of this folded area (will be the wide, non-numbered, yellow color bar of the pH strip). Remove the pH strip from the liquid and compare the color in the fold of the strip to the color chart on the pH strip. If there is not an exact color match with this scale, then estimate an intermediate value. Record the pH reading on the enclosed Microbiology Survey Reporting Form. Upon completion of this step, the blue capped tube and pH indicator paper may be discarded.
- Step 18. With the pH testing completed, now proceed with the Microbiology testing - Step 19.

Completion of Microbiology Testing

Inoculation of APBs Bottles

- Step 19. Number the **red-cap** bottles 1 through 5. (Located in the foam block).
- Step 20. Remove the metal tabs from the centers of all **red-cap** bottles. Do not remove the entire metal seal.
- Step 21. Using one of the enclosed alcohol swabs, wipe the exposed rubber part of the cap on each of the **red-cap** bottles.
- Step 22. Unwrap one of the four syringes. Draw one (1) ml of liquid sample from the “ADS” (orange-capped) tube into the first syringe.
- Step 23. Insert the syringe needle into the top of the first **red-cap** bottle and inject the sample into the bottle (**red-cap** bottle 1). Move the syringe plunger up and down a couple of times to mix the sample in the bottle. Empty the syringe into this bottle prior to removing the syringe.
- Step 24. Remove the empty syringe and set it aside. *You will not use it again.*
- Step 25. Unwrap the second of the four syringes. Insert it into the first **red-cap** bottle.
- Step 26. Now pull up on the syringe plunger to draw up one (1) ml of sample from the first **red-cap** bottle into the syringe.
- Step 27. Pull the syringe out of **red-cap** bottle 1, insert the needle into **red-cap** bottle 2, and inject the liquid into bottle 2. Once again, move the syringe plunger up and down to mix the sample, draw up one (1) ml of liquid, and inject it into **red-cap** bottle 3.
- Step 28. Repeat the above step until all of the **red-cap** bottles have been injected. Note: you do not have to draw any liquid out of the last **red-cap** bottle (bottle 5).
- Step 29. Remove the empty syringe from the last **red-cap** bottle and set the syringe aside. *You will not use it again.*

Inoculation of SRBs Bottles

- Step 30. Number the **green-cap** bottles 1 through 5. (Located in the foam block).
- Step 31. Remove the metal tabs from the centers of all **green-cap** bottles. Do not remove the entire metal seal.
- Step 32. Using one of the enclosed alcohol swabs, wipe the exposed rubber part of the cap on each of the **green-cap** bottles.
- Step 33. Unwrap the third of the four (4) syringes. Draw one (1) ml of liquid sample from the “ADS” (orange-capped) tube into the first syringe.
- Step 34. Insert the syringe needle into the top of the first **green-cap** bottle and inject the sample into the bottle (**green-cap** bottle 1). Move the syringe plunger up and down a couple of times to mix the sample in the bottle. Empty the syringe into this bottle prior to removing the syringe.
- Step 35. Remove the empty syringe and set it aside. *You will not use it again.*
- Step 36. Unwrap the fourth syringe. Insert it into the first **green-cap** bottle.
- Step 37. Now pull up on the syringe plunger to draw up one (1) ml of sample from the first **green-cap** bottle into the syringe.
- Step 38. Pull the syringe out of **green-cap** bottle 1, insert the needle into **green-cap** bottle 2, inject the liquid into bottle 2. Once again, move the syringe plunger up and down to mix the sample, draw up one (1) ml of liquid, and inject into **green-cap** bottle 3.
- Step 39. Repeat the above step until all of the **green-cap** bottles have been injected. Note: you do not have to draw any liquid out of the last **green-cap** bottle (bottle 5).
- Step 40. Remove the empty syringe from the last **green-cap** bottle and set the syringe aside. *You will not use it again.*

Step 41. Upon completion of Step 40, the “ADS” (orange-capped) tube and syringes may be discarded. The syringes are to be discarded per the section entitled “Syringe Disposal”. The “ADS” tube, alcohol swabs, etc. can be disposed of in the trash.

Recording the Microbiology Survey Results

- Step 42. The carton in which the Microbiology Survey APB and SRB bottles, syringes, etc. were originally shipped can be used as an incubator for the completion of the Microbiology Survey. After inoculating all the bacteria bottles, place the foam block containing the inoculated SRB and APB bottles back into the box, and close the lid. All other materials can then be discarded. The Microbiology Survey record found as a label on the lid of the box can be completed, for ease of reference.
- Step 43. Keep the bottles out of direct sunlight and at room temperature for one month (30 days).
- Step 44. Ten (10) days after inoculation, check the APB (**red-cap**) bottles for any color changes. If the liquid has changed from red to yellow, count that bottle as having changed (positive). Record the number of APB (**red-cap**) bottles, starting with Bottle 1, that have changed on the enclosed Microbiology Survey Reporting Form. After the ten (10) days, no further observations are needed for the APB (**red-cap**) bottles.
- Step 45. Twenty-eight (28) to thirty (30) days after inoculation, check the SRB (**green-cap**) bottles for any color changes. If the liquid has changed from clear to black, count that bottle as having changed (positive). Record the number of SRB (**green-cap**) bottles, starting with Bottle 1, that have changed on the enclosed Microbiology Survey Reporting Form. After the thirty (30) days, no further observations are needed for the SRB (**green-cap**) bottles.
- Step 46. After the results have been recorded on the Microbiology Survey Reporting Form, the APB and SRB bottles can be discarded in the trash. No special handling or disposal requirements are applicable for their disposal.

Syringe Disposal

Needles and syringe barrels are to be destroyed and disposed in accordance with Federal and Local laws. At a minimum, the needles must be destroyed before discarding by cutting the tips off the needle or by bending back the needle tips. Syringes must be destroyed by breaking or shattering the barrel.

(Company)

(Location)

MICROBIOLOGY SURVEY REPORTING FORM

Storage Field	Line Number	
Bell Hole Number	Well Number	Drip Name and Number
Township/District	County	State
Comments		
Sample Location		Sample Number
Sample Type <input type="checkbox"/> - Soil <input type="checkbox"/> - Scale <input type="checkbox"/> - Solids <input type="checkbox"/> - Sludge/Gunk <input type="checkbox"/> - Mud <input type="checkbox"/> - Slime <input type="checkbox"/> - MIC <input type="checkbox"/> - Pipeline Dust <input type="checkbox"/> - Other _____		
Comments		
Sample Collection Date	Sample Collection Time <input type="checkbox"/> - AM <input type="checkbox"/> - PM	Sampler
SAMPLE DESCRIPTION Color _____ Odor _____ Texture _____		
pH MEASUREMENT pH _____	WEATHER CONDITIONS Air Temperature _____ Conditions _____	
MICROBIOLOGY SURVEY RESULTS		
Positive Culture Bottles Bottle Number (Red Cap) Number of Days	APBs	_____ Bacteria Colonies/ml
	○ (1) ○ (2) ○ (3) ○ (4) ○ (5) _____ ⊗ Record color change from red to yellow after 10 days	
Positive Culture Bottles Bottle Number (Green Cap) Number of Days	SRBs	_____ Bacteria Colonies/ml
	○ (1) ○ (2) ○ (3) ○ (4) ○ (5) _____ ⊗ Record color change from clear to black after 28-30 days	
TESTED BY	DATE	
COMMENTS		

MICROBIOLOGY SURVEY

INTERPRETATION OF RESULTS

1. POSITIVE RESULTS ARE AS FOLLOWS:

<u>BACTERIA</u>	<u>MEDIA</u>	<u>POSITIVE</u>
APBs	Red	Media turns yellow
SRBs	Clear With Nail	Media turns dark gray to black

2. The number of bottles that show positive results in the interval time (10 days APBs and 28 to 30 days SRBs) can be used to calculate the bacteria level in the original sample by the following table:

<u>NUMBER OF POSITIVE BOTTLES</u>	<u>BACTERIA COLONIES/ML</u>	<u>REPORTING VALUE</u>
0	<1 - 10	<10
1	1 - 10	10
2	10 - 100	100
3	100 - 1,000	1,000
4	1,000 - 10,000	10,000
5	≥10,000 - 100,000	100,000

3. When a sample has elevated H₂S levels, the sulfate reducer (SRBs) nutrient bottle no. 1 will often turn “positive” (black) within 15 - 60 seconds of inoculation. This occurrence should be considered “no growth”, if only this bottle is positive after 28 days. If SRB bottle no. 2 turns black immediately, a new sample should be obtained and the H₂S purged from the sample before inoculation. If SRB bottle no. 2 turns black hours or days after inoculation, the bottle should be marked “positive” and the results recorded, per the table in Item 2.