COLONY SAMPLE PREP *and* PCR

1. Pick and transfer colony into a 1.5 mL microcentrifuge tube containing 500 µL of dH_2O.
2. Mix contents by pipetting sample up and down or vortexing.
3. Transfer 5 µL from 1.5 mL microcentrifuge tube generated in Step 2 to brewSTAT PCR tube.
    a. Note: Open brewSTAT PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
4. Place brewSTAT PCR tube in IS PCR Thermocycler, select "brewSTAT" program and start program as directed by the IS Thermocycler User Guide.
5. Upon completion of "brewSTAT" program, end program and proceed to Cassette Sample Analysis section Step 1.

CASSETTE SAMPLE ANALYSIS

1. Remove brewSTAT PCR tubes from IS PCR Thermocycler and add 4 drops of Buffer B directly to each brewSTAT PCR tube.
2. Transfer entire contents (~200 µL) of brewSTAT PCR tube directly to Veriflow® brewSTAT cassette sample window with pipette. A separate Veriflow® brewSTAT cassette must be used for each brewSTAT PCR Tube.
3. Allow Veriflow® brewSTAT test to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of Buffer B directly to each Veriflow® brewSTAT cassette sample window.
5. Allow Veriflow® brewSTAT test to develop for 1 minute ± 15 seconds.
    a. Note: Veriflow® brewSTAT cassette can be developed for up to 120 minutes before proceeding to Step 6.
6. Retract Veriflow® brewSTAT cassette switch and immediately record results.
    a. The appearance of one red line (control) in the Veriflow® brewSTAT cassette window indicates a negative result.
    b. The appearance of two red lines (control and test) in the Veriflow® brewSTAT cassette window indicates a positive result.
    c. If the control line fails to develop, the test is invalid and will need to be repeated.

CUSTOMER SERVICE

Invisible Sentinel customer service and technical assistance can be reached between 9AM and 5PM Eastern time by calling 215-966-6118 and asking for an Invisible Sentinel sales or technical representative. Training on this product and all Invisible Sentinel test kits is available.

MSDS INFORMATION AVAILABLE

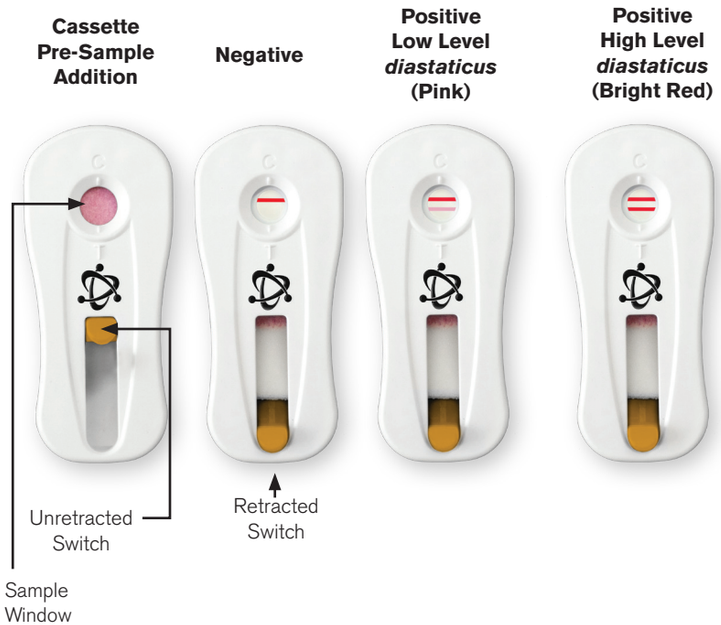
Material Safety Data Sheets (MSDS) are available for this test kit and all of Invisible Sentinel's test kits by calling Invisible Sentinel at 215-966-6118.

APPENDIX 1: ASSAY APPLICABILITY

Veriflow® brewSTAT has been validated for the detection of *Saccharomyces cerevisiae var diastaticus* in final brewery products and in-process brewing samples.

APPENDIX 2: RESULTS INTERPRETATION

The control line, as indicated by the letter C on the test cassette, should always develop. The test line, as indicated by the letter T on the cassette, will only develop in the event of a positive result for *Saccharomyces cerevisiae var diastaticus*. If the control line fails to develop, the test is invalid, and will need to be repeated.



APPENDIX 3: CONFIRMATION OF RESULTS

Presumptive positive samples can be confirmed by plating on selective media and colony PCR.

APPENDIX 4: DISPOSAL

Invisible Sentinel devices are for single use only. Decontaminate all surfaces, media and reagents and discard in accordance with local, state, and federal regulations.

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brewSTAT Powered by Veriflow®

THE POWER OF **MOLECULAR DIAGNOSTICS** IN THE PALM OF YOUR HAND®

ASSAY PRINCIPLES

Veriflow® brewSTAT is a molecular based assay for the qualitative detection of *Saccharomyces cerevisiae var diastaticus*, commonly known as *Saccharomyces diastaticus*. The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 minutes and can generate results in as little as 4 hours without DNA purification. The Veriflow® brewSTAT system eliminates the need for gel electrophoresis or fluorophore based detection of target amplification and does not require complex data analysis. Veriflow® brewSTAT provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

INTENDED USER

The Veriflow® brewSTAT system is intended for use by personnel familiar with basic sample collection and preparation techniques associated with spoilage organism detection during the brewing process. Veriflow® brewSTAT is specifically designed to be easy-to-use and eliminates the need for advanced training in molecular biology.

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MATERIALS PROVIDED

1. IS brewSTAT PCR Reagent – Cat. No. IS0540
2. IS Buffer ACB – Cat. No. IS0714
3. IS Buffer B – Cat. No. IS0702
4. IS Digest Tube – Cat. No. IS0712
5. IS Veriflow® brewSTAT Assay Cassette – Cat. No. IS0121

OTHER MATERIALS NEEDED

1. Centrifuge compatible with 50 mL conical tubes, capable of 1800 x g (rcf)
2. 50 mL Conical Tubes (capable of being centrifuged at 1800 x g)
3. Invisible Sentinel SimpliAmp PCR Thermocycler – Cat. No. ISTC002
4. Incubator that provides continuous and stable temperatures of 30-35°)
5. Pipettes and tips for 5 µL, 100 µL, 1000 µL, 10 mL and 25 mL transfer volumes
6. IS STAT Broth – Cat. No. IS0359 (500 mL, prepared) or IS0360 (5 L, prepared), for enrichment-based applications

STORAGE OF MATERIALS

The Veriflow® brewSTAT kit components, including cassettes and buffers (Buffers ACB and B) should be stored at room temperature (20-25°C). The Veriflow® brewSTAT PCR and Digest reagents should be stored at -20°C ± 2°C.

PRECAUTIONS

1. Assay users should observe standard microbiological practices and safety precautions when performing this assay.
2. Do not use Veriflow® brewSTAT assay components past indicated expiration date.
3. Deviations from the assay protocol may impact overall test performance.

BEER SAMPLE PREP *and* PCR (for presumptive diastaticus contamination*)

1. Transfer 25 mL of beer to a 50 mL conical tube.
2. Centrifuge 50 mL conical with sample at 1800 x g for 10 minutes.
3. Decant supernatant from 50 mL tube (be careful not to disturb pellet).
4. Resuspend pellet in 250 µL of IS Buffer ACB. Mix until pellet is no longer visible.
5. Transfer 100 µL from the 50 mL tube to a thawed Digest tube.
   a. Note: Open Digest tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
6. Place Digest tube from Step 5 in IS PCR cycler, select “DIGEST” and start program as directed by the IS Thermocycler User Guide.
7. Upon completion of “DIGEST” program, end program and remove Digest tube from IS PCR cycler.
8. Transfer 5 uL from Digest tube generated in Step 7 to brewSTAT PCR tube. IMPORTANT:
   a. DO NOT collect 5 µL from the bottom of the Digest tube or disturb settled contents of the Digest tube. DNA is present in solution at the top of the tube.
   b. Open PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
9. Place brewSTAT PCR tube in IS PCR Thermocycler, select “brewSTAT” and start program as directed by the IS Thermocycler User Guide.
10. At the completion of “brewSTAT” program, end program and proceed to Cassette Sample Analysis section Step 1.

* Includes super-attenuation, gushing and deformed cans

BEER ENRICHMENT SAMPLE PREP *and* PCR

1. Transfer 25 mL of beer to a 50 mL conical tube containing 25 mL of IS STAT Broth.
2. Incubate sample for 48 hours at 30-35°C with lid loosened.
   a. DO NOT enrich any sample for greater than 48 hours. Incubation times exceeding 48 hours may impact test performance.
3. After incubation, tighten lid and centrifuge 50 mL conical with sample at 1800 x g for 10 minutes.
4. Decant supernatant from 50 mL tube (be careful not to disturb pellet).
5. Resuspend pellet in 50 mL conical tube with 250 µL of IS Buffer ACB. Mix until pellet is no longer visible.
6. Transfer 100 µL from the 50 mL tube to a thawed Digest tube.
   a. Note: Open Digest tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
7. Place Digest tube from Step 6 in IS PCR Thermocycler, select “DIGEST” and start program as directed by the IS Thermocycler User Guide.
8. Upon completion of “DIGEST” program, end program and remove Digest tube from IS PCR Thermocycler.
9. Transfer 5 µL from Digest tube generated in Step 8 to brewSTAT PCR tube. IMPORTANT:
   a. DO NOT collect 5 µL from the bottom of the Digest tube or disturb settled contents of the Digest tube. Target DNA is present in solution at the top of the tube.
   b. Open PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
10. Place PCR tube in IS PCR Thermocycler, select “brewSTAT” and start program as directed by the IS Thermocycler User Guide.
11. At the completion of “brewSTAT” program, end program and proceed to Cassette Sample Analysis section Step 1.

FERMENTATION SAMPLE PREP *and* PCR (for presumptive diastaticus contamination*)

1. Transfer 10 mL of fermentation sample to a 50 mL conical tube.
2. Centrifuge 50 mL conical with sample at 1800 x g for 10 minutes.
3. Decant supernatant from 50 mL tube (be careful not to disturb pellet).
4. Resuspend pellet in 1mL of IS Buffer ACB. Mix until pellet is no longer visible.
5. Transfer 100 µL from the 50 mL tube to a thawed Digest tube.
   a. Note: Open Digest tube only when adding sample and promptly close after to avoid cross contamination between tubes.
6. Place Digest tube from Step 5 in IS PCR cycler, select “DIGEST” and start program as directed by the IS Thermocycler User Guide.
7. Upon completion of “DIGEST” program, end program and remove Digest tube from IS PCR cycler.
8. Transfer 5 µL from Digest tube generated in Step 7 to brewSTAT PCR tube. IMPORTANT:
   a. DO NOT collect 5 µL from the bottom of the Digest tube or disturb settled contents of the Digest tube. DNA is present in solution at the top of the tube.
   b. Open PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
9. Place brewSTAT PCR tube in IS PCR Thermocycler, select “brewSTAT” and start program as directed by the IS Thermocycler User Guide.
10. At the completion of “brewSTAT” program, end program and proceed to Cassette Sample Analysis section Step 1.

* Includes super-attenuation

FERMENTATION ENRICHMENT SAMPLE PREP *and* PCR

1. Transfer 5 mL of fermentation sample to a 50 mL conical tube containing 45 mL of IS STAT Broth.
2. Incubate sample for 48 hours at 30-35°C with lid loosened.
   a. DO NOT enrich any samples for greater than 48 hours. Incubation times exceeding 48 hours may impact test performance.
3. After incubation, homogenize sample and transfer 10 mL to a new 50 mL conical tube.
4. Centrifuge 50 mL conical with 10 mL sample at 1800 x g for 10 minutes.
5. Decant supernatant from 50 mL tube (be careful not to disturb pellet).
6. Resuspend pellet in 1 mL of IS Buffer ACB. Mix until pellet is no longer visible.
7. Transfer 100 µL from the 50 mL tube to a thawed Digest tube.
   a. Note: Open Digest tube only when adding sample and promptly close after to avoid cross contamination between tubes.
8. Place DIGEST tube from Step 7 in IS PCR Thermocycler, select “DIGEST” and start program as directed by the IS Thermocycler User Guide.
9. Upon completion of “DIGEST” program, end program and remove Digest tube from IS PCR Thermocycler.
10. Transfer 5 µL from Digest tube generated in Step 9 to brewSTAT PCR tube. IMPORTANT:
    a. DO NOT collect 5 µL from the bottom of the Digest tube or disturb settled contents of the Digest tube. DNA is present in solution at the top of the tube.
    b. Open PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
11. Place brewSTAT PCR tube in IS PCR Thermocycler, select “brewSTAT” and start program as directed by the IS Thermocycler User Guide.
12. At the completion of “brewSTAT” program, end program and proceed to Cassette Sample Analysis section Step 1.

YEAST SLURRY ENRICHMENT SAMPLE PREP *and* PCR

1. Transfer 1 mL of yeast slurry to a 50 mL conical tube containing 49 mL of IS STAT Broth.
2. Incubate sample for 48 hours at 30-35°C.
   a. DO NOT enrich any samples for greater than 48 hours. Incubation times exceeding 48 hours may impact test performance.
3. After incubation, homogenize sample and transfer 10 mL to a new 50 mL conical tube.
4. Centrifuge 50 mL conical with 10 mL sample at 1800 x g for 10 minutes.
5. Decant supernatant from 50 mL tube (be careful not to disturb pellet).
6. Resuspend pellet in 1 mL of IS Buffer ACB. Mix until pellet is no longer visible.
7. Transfer 100 µL from the 50 mL tube to a thawed Digest tube.
   a. Note: Open Digest tube only when adding sample and promptly close after to avoid cross contamination between tubes.
8. Place DIGEST tube from Step 7 in IS PCR Thermocycler, select “DIGEST” and start program as directed by the IS Thermocycler User Guide.
9. Upon completion of “DIGEST” program, end program and remove Digest tube from IS PCR Thermocycler.
10. Transfer 5 µL from Digest tube generated in Step 9 to brewSTAT PCR tube. IMPORTANT:
    a. DO NOT collect 5 µL from the bottom of the Digest tube or disturb settled contents of the Digest tube. DNA is present in solution at the top of the tube.
    b. Open PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
11. Place brewSTAT PCR tube in IS PCR Thermocycler, select “brewSTAT” and start program as directed by the IS Thermocycler User Guide.
12. At the completion of “brewSTAT” program, end program and proceed to Cassette Sample Analysis section Step 1.

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