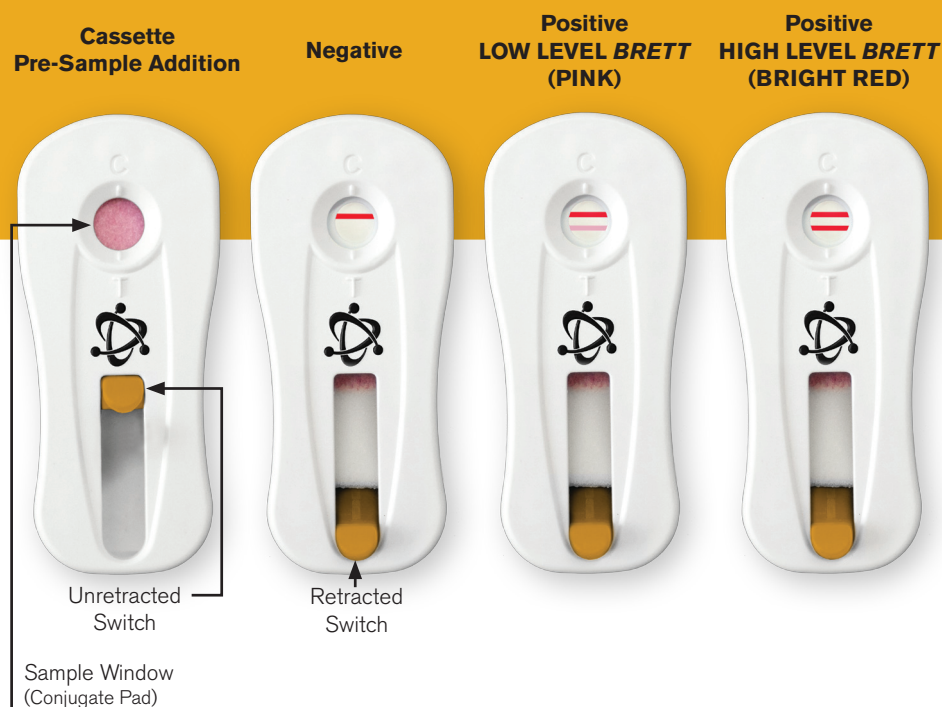


APPENDIX 1: ASSAY APPLICABILITY

brewBRUX[®] has been validated for the detection of *Brettanomyces bruxellensis* from final brewery products and samples from each step in the brewing process.



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MOLECULAR DIAGNOSTICS
IN THE PALM OF YOUR HAND[®]



APPENDIX 2: RESULTS INTERPRETATION

The control line, as indicated by the letter C on the *brewBRUX*[®] cassette, should always develop. The test line, as indicated by the letter T on the *brewBRUX*[®] cassette, will only develop in the event of a positive sample for *Brettanomyces bruxellensis*. 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 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.

ASSAY PRINCIPLES

brewBRUX[®] is a molecular based assay for the qualitative and quantitative detection of *Brettanomyces bruxellensis*. The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 minutes post PCR amplification, and generates results without enrichment or DNA purification. *brewBRUX*[®] eliminates the need for gel electrophoresis or fluorophore based detection of target amplification and provides same day results in under 4 hours. Ultimately, *brewBRUX*[®] provides the specificity and sensitivity of PCR based amplification in a cost-effective and easy-to-use format.

INTENDED USER

brewBRUX[®] is intended for use by personnel familiar with basic sample collection and preparation techniques associated with spoilage organism detection during fermentation and bottling. *brewBRUX*[®] is specifically designed to be easy-to-use and eliminates the need for advanced training in molecular biology.

Invisible Sentinel[®] and *Veriflow*[®] are trademarks of Invisible Sentinel, Inc., of Philadelphia, PA. U.S. Patent No. 8,183,059, 8,476,082 and patents pending. Purchase and use of this product is subject to Invisible Sentinel's Terms and Conditions of Sale located at www.invisiblesentinel.com.



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MATERIALS PROVIDED

1. IS BRETT PCR Tube – Cat. No. IS0508200
2. IS Buffer A – Cat. No. IS0701
3. IS Buffer B – Cat. No. IS0702
4. DIGEST Tube – Cat. No. IS0712
5. IS *brewBRUX*[®] Assay Cassette – Cat. No. IS0121

OTHER MATERIALS NEEDED

1. Invisible Sentinel SimpliAmp PCR Thermocycler – Cat. No. ISTC002
2. Centrifuge compatible with 50 mL conical tubes, capable of 1800 x g
3. Pipettes and tips capable of 5 µL, 100 µL, 1000 µL, and 25 mL volume transfers
4. 50 mL conical tubes (capable of being centrifuged at 1800 x g)

STORAGE OF MATERIALS

The *brewBRUX*[®] kit components, including cassettes and buffers (Buffer A and B) should be stored at room temperature (20-25°C). The BRETT PCR Tubes and IS DIGEST Tubes 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 *brewBRUX*[®] kit past indicated expiration date.
3. Deviations from the assay protocol may impact overall test performance.
4. Do not retract cassette switch until steps 1 through 5 of the Cassette Sample Analysis section has been completed as directed.

BEER SAMPLE PREP and PCR

1. Transfer 25 mL of beer to a 50 mL conical tube.
2. Centrifuge 50 mL conical tube with sample for 10 minutes at 1800 x g
3. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
4. Resuspend pellet in 50 mL conical tube with 1 mL of IS Buffer A. Mix until the pellet is no longer visible.
5. Transfer 100 µL from resuspended pellet in 50 mL conical tube generated in step 4 to 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 into IS PCR Thermocycler, select "DIGEST" and press "START RUN", as directed by the Thermocycler User Guide.
7. Upon completion of "DIGEST" program, press "STOP RUN," and remove **Digest** Tube from IS PCR Thermocycler.
8. Transfer 5 µL from **Digest** Tube generated in step 7 to BRETT PCR Tube.

IMPORTANT:

 - a. DO NOT collect 5 µL from the bottom of the **Digest** Tube or disturb settled contents of **Digest** Tube. Target DNA is present in solution at the top of the tube.
 - b. Open BRETT **PCR** Tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
9. Place BRETT **PCR** Tube into IS PCR Thermocycler, select "BRUXPCR" and press "START RUN", as directed by the Thermocycler User Guide.
10. Upon completion of "BRUXPCR" program, press "STOP RUN" and proceed to Cassette Sample Analysis section step 1.

ENRICHED BROTH (YM or BSB) SAMPLE PREP and PCR

1. Transfer 500 µL Buffer A to 1.5 mL microcentrifuge Tube.
2. Transfer 500 µL of enriched culture (YM or BSB) to tube generated in step 1.
3. Mix contents by pipetting sample up and down or by vortexing.
4. Transfer 5 µL of sample from step 3 to BRETT PCR Tube.
 - a. Note: Open BRETT PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
5. Place BRETT PCR Tube into IS PCR Thermocycler, select "BRUXPCR" and press "START RUN", as directed by the Thermocycler User Guide.
6. Upon completion of "BRUXPCR" program, press "STOP RUN," and proceed to Cassette Sample Analysis section step 1.

COLONY SAMPLE PREP and PCR

1. Pick and transfer colony into a 1.5 mL microcentrifuge tube containing 500 µL of dH₂O.
2. Mix contents by pipetting sample up and down or by vortexing.
3. Transfer 5 µL of colony re-suspension to **BRETT PCR** Tube.
 - a. Note: Open BRETT PCR Tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
4. Place BRETT PCR Tube into IS PCR Thermocycler and select "BRUXPCR" program and press "START RUN" as directed by the Thermocycler User Guide.
5. Upon completion of "BRUXPCR" program, press "STOP RUN" and proceed to Cassette Sample Analysis section step 1.

CASSETTE SAMPLE ANALYSIS

1. Remove tubes from IS PCR Thermocycler and add 4 drops of BUFFER B directly to each BRETT PCR Tube.
2. Transfer entire contents (200 µL) of BRETT PCR Tube directly to *brewBRUX*[®] cassette sample window with pipette. A separate *brewBRUX*[®] cassette must be used for each *brewBRUX*[®] PCR Tube.
3. Allow *brewBRUX*[®] cassette to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of BUFFER B directly to each *brewBRUX*[®] cassette sample window.
5. Allow *brewBRUX*[®] cassette to develop for 1 minute ± 15 seconds.
 - a. Note: *brewBRUX*[®] cassette can be developed for up to 120 min before proceeding to step 6.
6. Retract *brewBRUX*[®] cassette switch and immediately record results.
 - a. The appearance of one red line (control) in the *brewBRUX*[®] cassette sample window indicates a negative result.
 - b. The appearance of two red lines (control and test) in the *brewBRUX*[®] cassette sample window indicates a positive result.
 - i. Test line intensity indicates quantitative levels of *Brettanomyces bruxellensis*. (See Appendix 2 and provided signal reference card).

CUSTOMER SERVICE

Invisible Sentinel customer service and technical assistance can be reached Monday-Friday between 9AM and 5PM Eastern Standard 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.

