brewDEK
Powered by Veriflow®

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

## ASSAY PRINCIPLES

*brewDEK*® is a molecular based assay for the qualitative detection of *Dekkera* species. 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. *brewDEK*® eliminates the need for gel electrophoresis or fluorophore based detection of target amplification and provides same day results in under 4 hours. Ultimately, *brewDEK*® provides the specificity and sensitivity of PCR based amplification in a cost-effective and easy-to-use format.

## INTENDED USER

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



V. IS0974.1

INVISIBLE SENTINEL®

**Phone** 215.966.6118
**Email** info@invisiblesentinel.com | **www.invisiblesentinel.com**

INVISIBLE SENTINEL®

**Phone** 215.966.6118
**Email** info@invisiblesentinel.com | **www.invisiblesentinel.com**

## MATERIALS PROVIDED

1. IS DEK PCR tube – Cat. No. IS0524200
2. IS Buffer A – Cat. No. IS0701
3. IS Buffer B – Cat. No. IS0702
4. IS Digest Tube – Cat. No. IS0712
5. IS *brewDEK*® 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 *brewDEK*® kit components, including cassettes and buffers (Buffer A and B) should be stored at room temperature (20-25°C). The DEK PCR tubes and 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 *brewDEK*® 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 250 µL of IS Buffer A. Mix until the pellet is no longer visible.
5. Transfer 100 µL from the 50 mL conical tube to thawed Digest tube.
   a. Note: Open Digest tube only when adding sample and promptly close after, to avoid cross contamination between Digest tubes.
6. Place Digest tube from step 5 into IS PCR Thermocycler, select "DIGEST" and start program as directed by the Thermocycler User Guide.
7. Upon completion of "DIGEST" program, stop program and remove Digest tube from IS PCR Thermocycler.
8. Transfer 5 µL from Digest tube generated in Step 7 to DEK 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 DEK PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
9. Place DEK PCR Tube into IS PCR Thermocycler, select "brewDEK" and start program as directed by the IS Thermocycler User Guide.
10. Upon completion of "brewDEK" program, stop program and proceed to Cassette Sample Analysis section Step 1.

## YEAST SLURRY PREP and PCR

1. Transfer 5 mL of yeast slurry to a 50 mL conical tube containing 45 mL of $dH_2O$. Mix thoroughly by inverting 12-15 times until the mixture is homogeneous.
2. Centrifuge 50 mL conical tube with sample for 10 minutes at 20 x g.
3. Transfer the top 25 mL of supernatant to a new 50 mL conical tube.
4. Centrifuge 50 mL conical tube generated in step 3 above for 10 minutes at 1800 x g).
5. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
6. Resuspend pellet in 50 mL conical tube with 1 mL of Buffer A. Mix until the pellet is no longer visible.
7. Transfer 100 µL from 50 mL conical tube to a thawed Digest Tube.
   a. Note: Open Digest Tube only when adding sample and promptly close after, to avoid cross contamination between Digest tubes.
8. Place Digest tube from Step 7 in IS PCR Thermocycler, select "DIGEST" and press start program as directed by the 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 DEK 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 DEK PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
11. Place DEK PCR tube into IS PCR Thermocycler, select "brewDEK" program and start program as directed by the IS Thermocycler User Guide.
12. Upon completion of "brewDEK" program, end program 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 $dH_2O$.
2. Mix contents by pipetting sample up and down or by vortexing.
3. Transfer 5 µL of colony re-suspension to DEK PCR tube.
   a. Note: Open DEK PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
4. Place DEK PCR tube into IS PCR Thermocycler, select "brewDEK" program and start program as directed by the Thermocycler User Guide.
5. Upon completion of "brewDEK" program, stop program 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 DEK PCR tube.
2. Transfer entire contents (200 µL) of DEK PCR tube directly to *brewDEK*® cassette sample window with pipette. A separate *brewDEK*® cassette must be used for each DEK PCR tube.
3. Allow *brewDEK*® cassette to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of Buffer B directly to each *brewDEK*® cassette sample window.
5. Allow *brewDEK*® cassette to develop for 1 minute ± 15 seconds.
   a. Note: *brewDEK*® cassette can be developed for up to 120 minutes before proceeding to Step 6.
6. Retract *brewDEK*® cassette switch and record results.
   a. The appearance of one red line (control) in the *brewDEK*® cassette sample window indicates a negative result.
   b. The appearance of two red lines (control and test) in the *brewDEK*® cassette sample window indicates a positive result.

## 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

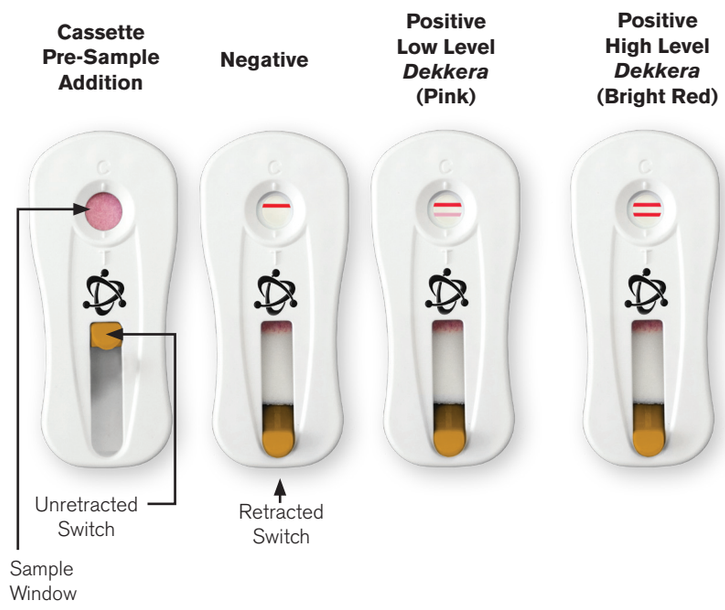
Material Safety Data Sheets (MSDS) are available for this test kit and all of Invisible Sentinel's test kits by calling Invisible Sentinel at 215-966-6118.

## APPENDIX 1: ASSAY APPLICABILITY

*brewDEK*® has been validated for the detection of ***Dekkera (bruxellensis, naardenensis, anomalus and custersianus)*** species in final brewery products and from samples generated during the brewing process.

## APPENDIX 2: RESULTS INTERPRETATION

The control line, as indicated by the letter C on the *brewDEK*® cassette, should always develop. The test line, as indicated by the letter T on the *brewDEK*® cassette, will only develop in the event of a positive result for *Dekkera* species. 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.

INVISIBLE SENTINEL®