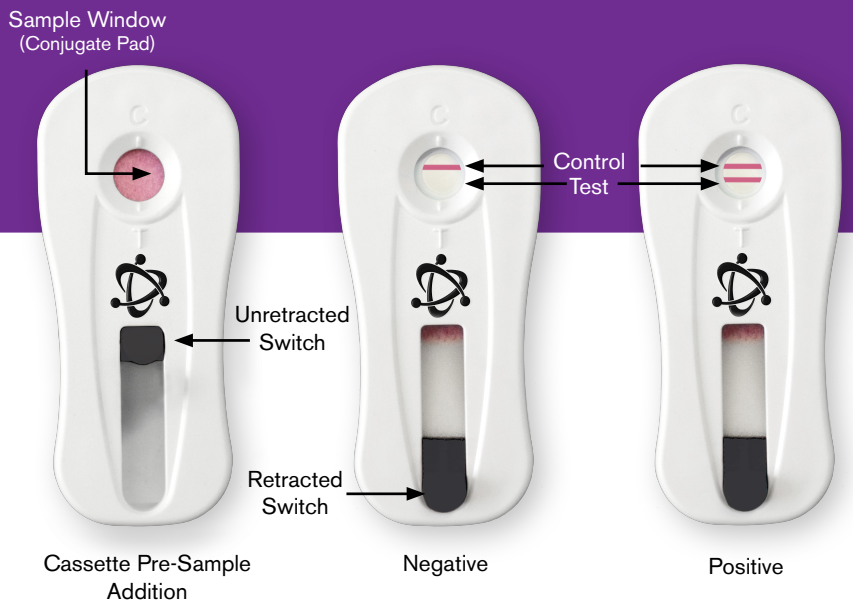
APPENDIX 1: ASSAY APPLICABILITY

Veriflow® DEK has been validated for the detection of Dekkera (bruxellensis, naardenensis, anomala and custersianus) species in beverage and environmental matrices and from beverage samples.

veriFLOW®
Dekkera species



APPENDIX 2: RESULTS INTERPRETATION

The control line, as indicated by the letter C on the Veriflow® DEK cassette, should always develop. The test line, as indicated by the letter T on the Veriflow® DEK cassette, will only develop in the event of a positive sample 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.

V.IS0987.1

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

ASSAY PRINCIPLES

Veriflow® DEK is a molecular based assay for the qualitative detection of Dekkera species in beverage and environmental samples. The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 minutes post PCR amplification and requires 48-72 hours of incubation for maximum sensitivity. The Veriflow® DEK system eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Ultimately, Veriflow® DEK provides the specificity and sensitivity of PCR based amplification in a cost-effective and easy-to-use format.

INTENDED USER

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

INVISIBLE SENTINEL®

Phone 215.966.6118 | **Fax** 215.921.5091
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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. IS0712200
5. IS Veriflow® DEK Assay Cassette – Cat. No. IS0101

MATERIALS PURCHASED SEPARATELY

1. Yeast Mold (YM) Broth – Cat. No. IS0340

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)
5. Sample enrichment bags
6. Sampling sponges or swabs
7. 1.5 mL microcentrifuge tube (for colony pick protocol)
8. dH_2O

STORAGE OF MATERIALS

The Veriflow® DEK kit components, including cassettes, growth media 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 Veriflow® DEK 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.

1x YM MEDIA PREPARATION for ENVIRONMENTAL SAMPLES

1. Add 21 grams IS YM Broth media per 1 Liter dH_2O and autoclave for 15 minutes at 121°C.
2. Allow media to cool to room temperature (20-25°C) before use.
3. Media can be stored at room temperature for up to 30 days.

2x YM MEDIA PREPARATION for BEVERAGE SAMPLES

1. Add 42 grams IS YM Broth media per 1 Liter dH_2O and autoclave for 15 minutes at 121°C.
2. Allow media to cool to room temperature (20-25°C) before use.
3. Media can be stored at room temperature for up to 30 days.

ENVIRONMENTAL SAMPLE PREP and PCR

1. Sample designated area with sponge/swab and place in sample enrichment bag.
2. Add 50 mL of 1x YM broth to the bag and briefly massage the sponge to homogenize sample.
3. Incubate at 25° C ± 2°C for 48 hours.
4. Transfer 50 mL from sample enrichment bag to a 50 mL conical tube.
5. Centrifuge 50 mL conical tube with sample for 10 minutes at 1800 x g.
6. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
7. Resuspend pellet in 50 mL conical tube with 100 µL of IS Buffer A. Mix until the pellet is no longer visible.
8. Transfer 100 µL from 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.
9. Place Digest Tube from step 8 into IS PCR Thermocycler, select "DIGEST DEK" and press "START RUN", as directed by the Thermocycler User Guide.
10. Upon completion of "DIGEST DEK" program, press "STOP RUN" and remove Digest Tube from IS PCR Thermocycler.
11. Transfer 5 µL from Digest Tube generated in step 10 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.
12. Place DEK PCR Tube into IS PCR Thermocycler, select "VFLOWDEK" program and press "START RUN" as directed by the Thermocycler User Guide.
13. Upon completion of "VFLOWDEK" program, press "STOP RUN", and proceed to Cassette Sample Analysis section step 1.

BEVERAGE SAMPLE PREP and PCR

1. Combine 100 mL of beverage sample with 100 mL of 2X YM broth in sample enrichment bag.
2. Incubate at 25° C ± 2°C for 72 hours (48 hours is sufficient for preservative-free beverage products).
3. Transfer 50 mL from sample enrichment bag to a 50 mL conical tube.
4. Centrifuge 50 mL conical tube with sample 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 100 µL of IS Buffer A. Mix until the pellet is no longer visible.
7. Transfer 100 µL from 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.
8. Place Digest Tube from step 7 into IS PCR Thermocycler, select "DIGEST DEK" and press "START RUN", as directed by the Thermocycler User Guide.
9. Upon completion of "DIGEST DEK" program, press "STOP RUN" and remove Digest Tube from IS PCR Thermocycler.
10. Transfer 5 µL from Digest Tube generated in step 9 to DEK PCR Tube.
    IMPORTANT:
    c. 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.
    d. 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 "VFLOWDEK" program and press "START RUN" as directed by the Thermocycler User Guide.
12. Upon completion of "VFLOWDEK" 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 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 "VFLOWDEK" program and press "START RUN" as directed by the Thermocycler User Guide.
5. Upon completion of "VFLOWDEK" program, press "STOP RUN", and proceed to Cassette Sample Analysis section step 1.

CASSETTE SAMPLE ANALYSIS

1. Remove DEK PCR 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 Veriflow® DEK cassette sample window with pipette. A separate Veriflow® DEK cassette must be used for each DEK PCR Tube.
3. Allow Veriflow® DEK cassette to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of Buffer B directly to each Veriflow® DEK cassette sample window.
5. Allow Veriflow® DEK cassette to develop for 1 minute ± 15 seconds.
    a. Note: Veriflow® DEK cassette can be developed for up to 120 min before proceeding to step 6.
6. Retract Veriflow® DEK cassette switch and record results.
    a. The appearance of one red line (control) in the Veriflow® DEK cassette sample window indicates a negative result.
    b. The appearance of two red lines (control and test) in the Veriflow® DEK 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.