



THE POWER OF MOLECULAR DIAGNOSTICS IN THE PALM OF YOUR HAND[®]

ASSAY PRINCIPLES

The *Lactobacillus* and *Pediococcus* Viability Kit is used in tandem with the Veriflow[®] and veriPRO[®] *Lactobacillus* and *Pediococcus* molecular based assays (brewPAL[®], brewLAP[®] and brewPRO) for the detection of viable *Lactobacillus* and *Pediococcus* species capable of causing spoilage in brewery products. The Viability Kit utilizes a proprietary dye, PMAxx*, which selectively enters and binds the DNA of non-viable cells, preventing PCR amplification of these bacteria. Ultimately, the Viability Kit allows users to differentiate between viable and non-viable bacteria post-pasteurization using the brewPAL[®], brewLAP[®] and brewPRO assay systems.

INTENDED USER

The *Lactobacillus* and *Pediococcus* Viability Kit is intended for use by personnel familiar with basic sample collection and preparation techniques associated with spoilage organism detection during production and packaging. The Viability Kit is specifically designed to be easy-to-use and eliminate the need for advanced training in molecular biology.

*Invisible Sentinel[®], Veriflow[®], brewPAL[®], brewLAP[®], and veriPRO[®] are registered trademarks of Invisible Sentinel, Inc, of Philadelphia, PA. U.S. Patent No. 8,183,059 and other patents pending. Purchase and use of this product is subject to Invisible Sentinel's Terms and Conditions of Sale located at <http://www.invisiblesentinel.com>. *PMAxx is a registered trademark of Biotium, Inc.*

V. IS2276.0



Phone 215.966.6118

Email info@invisiblesentinel.com | www.invisiblesentinel.com



Phone 215.966.6118

Email info@invisiblesentinel.com | www.invisiblesentinel.com

MATERIALS PROVIDED

1. IS Buffer Q – Cat No. IS0715
2. 8 black microcentrifuge tubes containing 100 µL Viability Solution – Cat No. IS0995
3. 8 clear (transparent) microcentrifuge tubes – Cat No. IS0996

MATERIALS NEEDED

1. IS LED Photolysis Device – Cat No. ISTC003
2. Pipettes and tips for 5, 100, 200, 500 µL volumes
3. 50 mL conical tubes (capable of withstanding 3000 x g centrifuge speed)
4. Centrifuge compatible with 50 mL conical tubes, capable of 3000 x g
5. Microcentrifuge compatible with 1.5 mL conical tubes, capable of 3000 x g
6. Vortex (optional)
7. brewPAL® (IS1020200), brewLAP® (IS1042200), or brewPRO (IS1063LC or IS1063QS) kit
8. End-over-end mixer for 1.5 mL tubes

STORAGE OF MATERIALS

The Viability Kit, Buffer Q and clear tubes should be stored at room temperature (20-25°C). Black microcentrifuge tubes containing viability solution and PCR 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 the Viability Kit past indicated expiration date.
3. Deviations from the assay protocol may impact overall test performance.

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.

SDS INFORMATION AVAILABLE

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

VIABILITY SAMPLE PREP AND PCR – Performed with brewPAL®, brewLAP® and brewPRO kit components

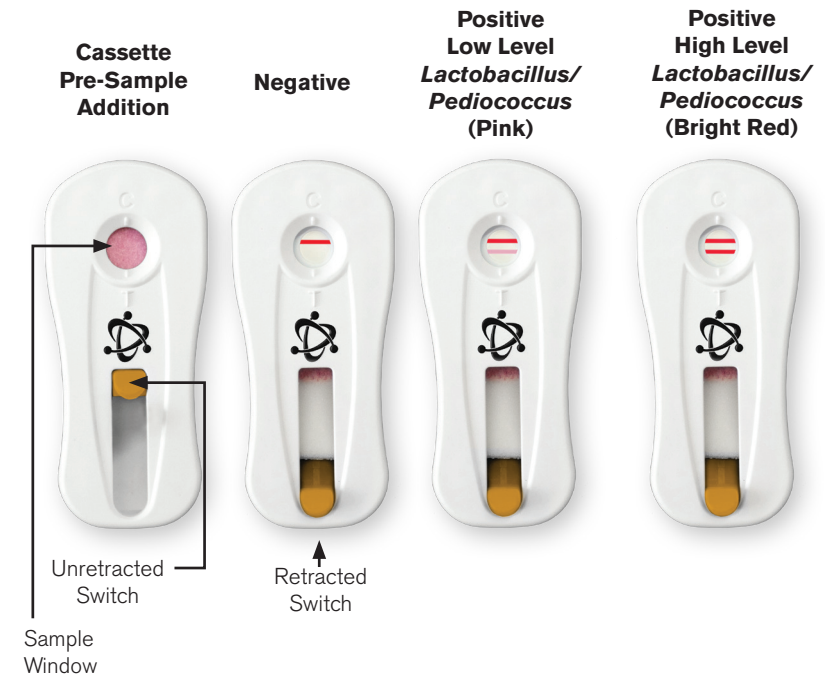
1. Transfer 25 mL of beer sample to a 50 mL conical tube.
2. Centrifuge 50 mL conical tube with sample for 10 minutes at 3000 x g.
3. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
4. Resuspend pellet in 50 mL conical tube with 250 µL of Buffer Q. Mix until the pellet is no longer visible.
5. Transfer 500 µL from resuspended pellet in 50 mL conical tube generated in Steps 1-4 to a black microcentrifuge tube containing 100 µL of Viability Solution.
6. Incubate treated sample for 5 minutes at room temperature on a mix using an end-over-end mixer.
7. Transfer sample to clear microcentrifuge tube and incubate sample for 10 minutes in LED Photolysis Device.
8. Add 500 µL of Buffer Q and centrifuge sample at 3000 x g for 10 minutes in microcentrifuge.
9. Decant supernatant and resuspend pellet in 100 µL of Buffer Q.
10. Transfer 5 µL of the resuspension from Step 9 to a thawed brewPAL®/brewLAP®/brewPRO PCR tube.
 - a. Note: Open brewPAL®/brewLAP®/brewPRO PCR tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
11. Place brewPAL®/brewLAP®/brewPRO PCR tube into a thermocycler and initiate appropriate program as directed by each assay product insert.
12. Upon completion of PCR program, stop the run and proceed to Cassette Sample Analysis (not applicable for brewPRO system).

CASSETTE SAMPLE ANALYSIS – Performed with brewPAL® and brewLAP® kit components

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

APPENDIX 1: RESULTS INTERPRETATION

If running brewPAL® or brewLAP®; the control line, as indicated by the letter C on the Veriflow® cassette, should always develop. The test line, as indicated by the letter T on the Veriflow® cassette, will only develop if the respective target is detected. If the control line fails to develop, the test is invalid, and will need to be repeated.



APPENDIX 2: ASSAY APPLICABILITY

The *Lactobacillus* and *Pediococcus* Viability Kit has been validated for the detection of viable *Lactobacillus* and *Pediococcus* species capable of causing spoilage in beer. It is intended to be used on post-pasteurized beer samples.

APPENDIX 3: 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.