brew PRO
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THE POWER OF
MOLECULAR DIAGNOSTICS
IN THE PALM OF YOUR HAND®

ASSAY PRINCIPLES

brewPRO is a real-time PCR assay for the detection of *Lactobacillus* and *Pediococcus* species capable of causing spoilage in brewery products. The assay utilizes a multiplex detection method which targets total *Lactobacillus* and *Pediococcus* on the FAM channel, hops resistance plasmids enabling *Lactobacillus* and *Pediococcus* survival in the presence of inhibitory hops compounds on the ROX channel, and an internal amplification control (IAC) on the HEX channel. *brewPRO* couples the advantages of the real-time format with a streamlined sampling protocol eliminating the need for DNA purification and provides same day results in under 3 hours.

INTENDED USER

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

V. IS2274.0

INVISIBLE SENTINEL®

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

MATERIALS PROVIDED

1. IS *brewPRO* PCR Reagent – Cat No. IS0541
2. IS Buffer A – Cat. No. IS0701

MATERIALS NEEDED

1. Roche LC480 II Real-Time PCR Instrument or comparable instrument capable of detecting FAM, HEX, and ROX fluorophores.
2. Centrifuge compatible with 50 mL conical tubes, capable of 3000 x g.
3. Pipettes and tips capable of 5 µL and 100-500 µL volume transfers.
4. 50 mL conical tubes (capable of withstanding 3000 x g centrifuge speed).

MATERIALS NEEDED (ENRICHMENT METHOD)

1. NBB-PCR broth (e.g. Dohler Cat No. 7.85420.782 or equivalent).
2. Centrifuge compatible with 1.5 mL centrifuge tubes (capable of withstanding 3000 x g centrifuge speed).
3. 15 mL conical tubes and 1.5 mL centrifuge tubes.

STORAGE OF MATERIALS

The *brewPRO* Buffer A should be stored at room temperature (20°-25°C). The brew*PRO* PCR 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 *brewPRO* kit past indicated expiration date.
3. Deviations from the assay protocol may impact overall test performance.

BEER SAMPLE PREP and PCR

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 A. Mix until the pellet is no longer visible.
5. Transfer 5 µL from resuspended pellet in 50 mL conical tube generated in Step 4 to *brewPRO* PCR tube.
    a. Note: Open *brewPRO* PCR tube only when adding sample and promptly close after to avoid cross contamination between tubes.
6. Initiate program as outlined in Appendix 1.

BEER ENRICHMENT METHOD and PCR

1. Transfer 5 mL of beer sample to a 15 mL conical tube containing 5 mL of NBB-PCR Broth.
2. Cap conical tube generated in Step 1, invert gently and incubate for 24-48 hours at 30-32° C under anaerobic conditions..
    a. Note: Leave 15 mL conical tube cap slightly loose (1/4 to 1/2 turn) to enable efficient gas exchange.
3. Remove conical tube from incubator and cap tightly. Homogenize by inverting.
4. Transfer 1 mL of enrichment to a 1.5 mL microcentrifuge tube.

5. Centrifuge microcentrifuge tube with sample for 10 minutes at 3000 x g.
6. Remove supernatant from the microcentrifuge tube by pipetting (be careful not to disturb pellet.)
7. Resuspend pellet in microcentrifuge tube with 100 µL of Buffer A. Mix until the pellet is no longer visible.
8. Transfer 5 µL from resuspended pellet in microcentrifuge tube generated in Step 7 to brewPRO PCR tube.
    a. Note: Open brewPRO PCR tube only when adding sample and promptly close after to avoid cross contamination between tubes.
9. Initiate program as outlined in Appendix 1.

COLONY SAMPLE PREP and PCR

1. Pick and transfer colony into a 1.5 mL microcentrifuge tube containing 500 µL of dH$_2$O.
2. Mix contents by pipetting sample up and down or by vortexing.
3. Transfer 5 µL of colony re-suspension to *brewPRO* PCR tube.
    a. Note: Open brewPRO PCR tube only when adding sample and promptly close after to avoid cross contamination between tubes.
4. Initiate program as outlined in Appendix 1.

Appendix 1: Roche LC 480II REAL-TIME PCR USER GUIDE

1. Place brewPRO PCR tube into the LC480 II using the LightCycler 8-Tube Strip Adapter Plate.
2. Select "New Experiment from Template" in the LC480 II Overview window and choose the brewPRO program.
3. Enter sample ID's into the Sample Editor module, utilizing the Sample Subset module to indicate wells, if desired.
4. Save the experiment and hit "START RUN" in the Experiment module.
5. Once the run is complete, proceed to the Analysis module. Create a New Analysis by selecting "Abs Quant/2nd Derivative Max" from the list of analysis options provided. If samples are organized as a subset, select the appropriate sample subset from the dropdown menu.
6. Apply color compensation to eliminate signal crosstalk by selecting "In Database" from the Color Comp dropdown menu. Select the brewPRO Color Compensation object from the database. Once prompted, ensure that the FAM, HEX, and ROX channels are selected.
7. Press "Calculate" to obtain the Ct/Cp values. Inspect the traces for the characteristic exponential amplification curve shape (see Appendix 3).
8. The sample Ct/Cp results table from each channel can be exported as a text file by right clicking on the sample results table. These files can be opened in Microsoft Excel to record, organize and evaluate the data.
9. Perform Step 7-8 for each channel by toggling to each individual filter using the "Filter Comb" Button.
10. The *Lactobacillus* and *Pediococcus* score (PALscore) risk assessment can be performed on positive samples by pasting the FAM and ROX Ct/Cp values for a given sample into the PALscore Calculator excel sheet provided. The PALscore risk assessment evaluates the fold increase of hops resistance plasmids (ROX) relative to the genomic target copy number (FAM) as an indicator of the relative risk of spoilage.

APPENDIX 2: ASSAY APPLICABILITY

brewPRO has been validated for the detection of *Lactobacillus* and *Pediococcus* species capable of causing spoilage in beer. It is intended to be used on final brewery products and samples from each step in the brewing process.

APPENDIX 3: RESULTS INTERPRETATION

Amplification Curves
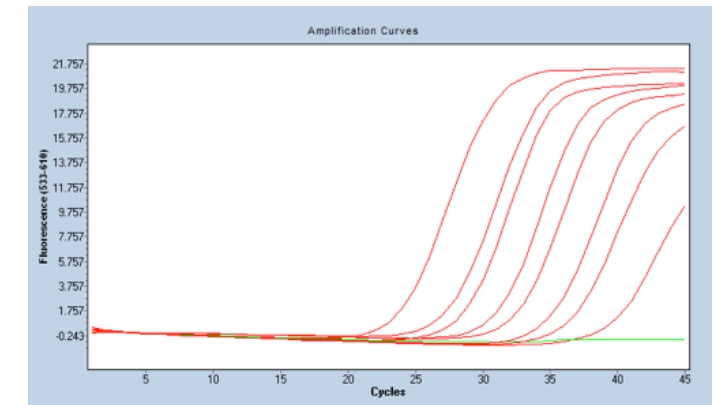
Amplification curves have a characteristic shape consisting of an initial lag phase, exponential amplification phase and a final plateau phase. The final plateau phase, which represents a decrease in reaction efficiency as reagents are consumed, may not be reached in reactions containing low levels of *Lactobacillus* or *Pediococcus*. Amplification curves that deviate from the characteristic shape should be interpreted with caution. For each brewPRO reaction, the cycle at which fluorescence signal rises above background fluorescence is determined and is called the "threshold cycle" (Ct) or "crossing point" (Cp), depending on the instrument. The Ct/Cp will occur at an earlier cycle for samples containing high levels of *Lactobacillus* or *Pediococcus* and will be delayed for reactions containing low levels of *Lactobacillus* or *Pediococcus*. The FAM channel is designed to detect total *Lactobacillus* or *Pediococcus* spoilage organisms by targeting genomic DNA. The ROX channel detects the presence of horA and horC hops resistance genes that are key determinants for beer spoilage ability in the presence of inhibitory hops compounds. A *Lactobacillus* and *Pediococcus* spoilage risk score (PALscore) can be generated by using the PALscore Calculator that compares the FAM and ROX Ct/Cp values to estimate the abundance of hops resistance genes relative to the genomic target. The HEX channel serves as an internal amplification control (IAC) to indicate a successful PCR reaction and should be detected at a Ct/Cp value between ~31-33 cycles.

APPENDIX 4: CONFIRMATION OF RESULTS

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

APPENDIX 5: DISPOSAL

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

CUSTOMER SERVICE

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