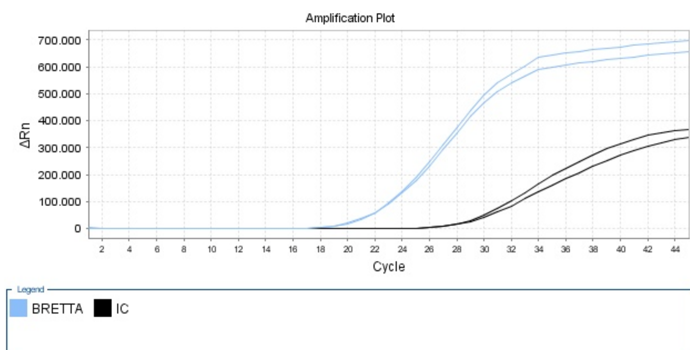
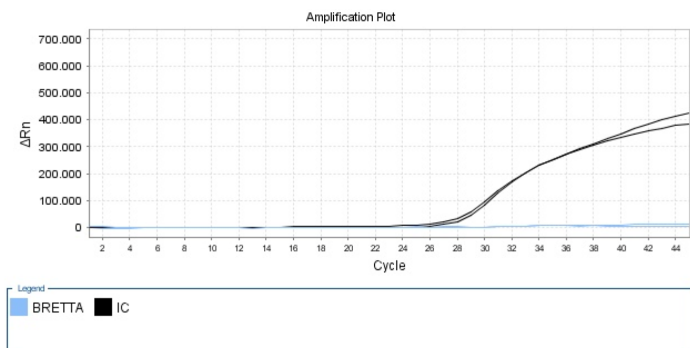
Positive Control –



Negative Control –



12. SPECIFICITY/INCLUSIVITY

a) 100 % Exclusivity, determined using 34 strains of closely related organisms or occurring in the same habitat.
b) 100 % Inclusivity, determined in 3 strains of *Brettanomyces/ Dekkera bruxellensis*.

13. SENSITIVITY

The REAL TIME DETECTION KIT *Brettanomyces/ Dekkera bruxellensis* detects 10^2-10^3 cfu/45mL after sample concentration. This kit has a reaction sensitivity of 20 fg of target DNA.

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BPMR is certified ISO 9001:2015

BIOPREMIER

REAL TIME DETECTION KIT

Brettanomyces/ Dekkera bruxellensis

Ref: BIOPFS-0019

1. PRODUCT DESCRIPTION

Beverages detection kits provide a simple, reliable, and rapid procedure for detecting the presence of a specific pathogen. The assay is based on 5’ nuclease real time PCR reactions to amplify a unique genomic sequence in the target microorganism.

2. TECHNOLOGY DESCRIPTION

PCR is a method used to amplify a specific DNA sequence in a reaction containing among other components, a thermostable DNA polymerase, nucleotides, and primers complementary to the target sequence. The DNA molecule denatures when this solution is heated, separating into two strands. As the solution cools, the primers anneal to the target sequences in the separated DNA strands and the DNA polymerase synthetizes a new strand by extending the primers with nucleotides, creating a copy of the DNA sequence (amplicons). When repeated, this cycle of denaturing, annealing, and extending exponentially increases the number of target amplicons. In Real Time PCR, the signal is measured in each cycle, using in most cases specific fluorescent probes. Fluorescence is measured by a detector and the associated software plots the fluorescence intensity versus the number of cycles, allowing the determination of the presence or absence of the target organism.

3. SCOPE

The yeast *Brettanomyces bruxellensis* (teleomorph *Dekkera bruxellensis*) is a spoilage yeast found in wine and various alcoholic beverages and can be found on grape berries, cellar equipment and barrels. *B. bruxellensis* has been described as the principal agent responsible for the formation of volatile phenols namely 4-ethylphenol and 4-ethylguaiacol which leads to the characteristic “Brett” taints associated with red wine, developed during wine ageing and bottling. At this stage, the wines are poorly protected via the appropriate use of fungicides and due to the resistance of *B. bruxellensis* to sulphur dioxide control of these yeasts becomes difficult. Moreover, *B. bruxellensis* viability in beverages seems to be enhanced by its ability to use alternative carbon sources, therefore being able to survive in the beverages long after fermentation. Detection of *B. bruxellensis* in beverages is usually performed using traditional microbiological techniques, based on culturing the organism on appropriate media and incubation for 1–2 weeks, which can have important economic consequences to wine and alcoholic beverages industry. Thus, in vitro amplification of DNA by polymerase chain reaction (PCR), in particular using real time PCR and specific fluorescent probes, is a powerful and reliable alternative for *B. bruxellensis* detection, providing results in much shorter times and allowing more efficient control procedures.

4. INTERNAL CONTROL (IC)

Beverages detection kits include an Internal Control (IC) in the master mix. This control is amplified at the same time as the target DNA sequence, but using a different set of primers and a probe labeled with a different fluorophore. The inclusion of the IC in each reaction allows the evaluation of PCR inhibitors in negative results.

5. APPLICATION

REAL TIME DETECTION KIT *Brettanomyces/ Dekkera bruxellensis* is intended for the rapid detection of *B. bruxellensis* in wine and other beverages after DNA extraction. It can also be used with other environmental samples. The kit was validated in the instruments ABI PRISM® 7500 Fast and ThermoScientific® PikoReal. The kit is compatible with all thermocyclers working in **FAM** and **ROX** channels. The detection kit must not be used for diagnostic procedures. For Research use only.
The procedure includes the following main steps:

Sample Concentration → DNA extraction → PCR set-up and run → Results and Interpretation

6. CONTENTS AND STORAGE

The kit contains reagents for 100 assays

CONTENTS	UNITS	COMPOSITION
Master Mix (blue cap)	2 tubes (2 x 840 µl)	Buffer, dNTPs, DNA polymerase
Assay Mix (brown cap)	1 tube (1 x 210 µl)	Primers and Probes
Negative Control (clear cap)	1 tube (1 x 70 µl)	Nuclease-free water
Positive Control (red cap)	1 tube (1 x 70 µl)	Target DNA

Store all contents at -20ºC and protect them from light as excessive exposure to light may affect the fluorescent probes. Minimize freeze-thaw cycles. Reagents stored as recommended may be used until the expiration date indicated in the tube.

7. MATERIAL REQUIRED AND NOT SUPPLIED

- Microcentrifuge
- Laminar Air Flow Cabinets/PCR Cabinets
- Disposable powder-free gloves
- Micropipettes and nuclease-free filter tips
- Real-time PCR instrument
- Tubes/Strips/Multiwell plates and accessories specific for each Instrument
- Lysis buffer / DNA extraction kit (example: BIOPEXT-0400)

8. PRECAUTIONS AND RECOMMENDATIONS

Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results, especially due to sample contamination or degradation of the nucleic acids contained in the samples. It is strongly recommended to have dedicated areas, materials and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures. Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area.
The user should always pay attention to the following:

- Read all the instructions provided before running the assay.
- Do not mix reagents from different batches.
- Wear proper PPE, including disposable gloves and laboratory coats.
- Store and extract positive material separately from all other reagents.

9. QUALITY CONTROL

In accordance with BPMR's ISO 9001, each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

10. PROCEDURE

10.1 Sample preparation

Beverages samples shall be concentrated by centrifugation or filtration

10.2 DNA extraction

1. Follow the instructions of BIOPREMIER DNA Rapid Extraction Buffer (ref: BIOPEXT-0400) (not included) or other kit or suitable protocol for DNA extraction.

10.3 PCR preparation

A – PCR mix

Always wear gloves for all PCR procedures.

1. Thaw the kit solutions. Mix thoroughly and centrifuge briefly
2. Prepare the reactions, as described below:

CONTENTS	Nº OF SAMPLES	
qPCR reaction	**1**	**10 (10 + 1)**
Master Mix	16 µl	176 µl
Assay Mix	2 µl	22 µl
Total Volume	**18 µl**	**198 µl**

Note: Prepare the PCR reaction for each sample, or in the alternative, prepare a Master Mix for the total number of reactions plus 10%, to cover pipetting losses (e.g. for 10 samples, prepare a volume for 11). In this case, prepare the Mix in a 1,5mL sterile, nuclease-free tube. Include 2 PCR reactions for the Positive and Negative controls.

3. Mix the prepared Mix by inverting the tube and centrifuge briefly
4. Dispense 18 µL aliquots of prepared Mix into the plate wells or PCR tube
5. For the negative control, pipette 2 µL of Negative Control tube (Clear Cap); Pipette 2 µL of DNA sample per well; and for the positive control, pipette 2 µL of Positive Control tube (Red Cap). Each PCR tube / well should have a final PCR volume of 20 µL
6. Centrifuge briefly the plate wells or PCR tubes
7. Place the reactions into the Real Time PCR instrument.

B – Program set up

Prepare the Real-Time PCR instrument according to the following temperature/time program:

PHASE	STEP	TEMPERATURE	TIME	ACQUISITION
Holding Stage	Step 1	50ºC	2 min.	No
	Step 2	95ºC	5 min.	No
Amplification - 45 cycles	Step 1	95ºC	30 sec.	No
	Step 2	56ºC	30 sec.	Yes
	Step 3	72ºC	30 sec.	No

TARGET	CHANNELS
Detection of *B. bruxellensis*	FAM
Detection of Internal Control (IC)	ROX

11. DATA ANALYSIS

For analysis of PCR results, select fluorescence display options. Samples with positive Ct-values are considered positive.
Important: Please, also check amplification curves, not only Ct values. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control. Adjust the Threshold, if necessary. Assessment of sample results should be performed after the positive and negative controls have been examined and determined to be valid. If the results of controls are not valid, the sample results cannot be interpreted.
Interpretation of PCR data:

a) Controls

To validate the assay, the controls must have the following results:

	***B. bruxellensis* DETECTION FAM**	**IC DETECTION ROX**
Negative Control	Negative	Positive
Positive Control	Positive	Positive

Note that if the controls do not match these results, the experiment must be repeated.

b) Samples

Interpretation of sample results is summarized in the following table:

***B. bruxellensis* DETECTION FAM**	**IC DETECTION ROX**	**INTERPRETATION**
Positive	Positive/Negative**	DNA detected, sample Positive for *B. bruxellensis*
Negative	Positive	No DNA detected, sample Negative for *B. bruxellensis*
Negative	Negative	Invalid Result*

*When both *B. bruxellensis* and IC detection are Negative, this means the presence of PCR inhibitors in the sample. Dilute the DNA sample (10-fold dilution) or perform another DNA extraction and repeat qPCR.
** High DNA concentration of the target in the sample can lead to a reduced or absent fluorescence signal of the IC.