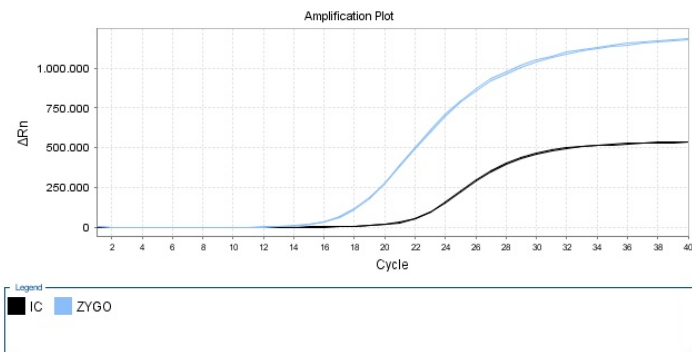


REAL TIME DETECTION KIT

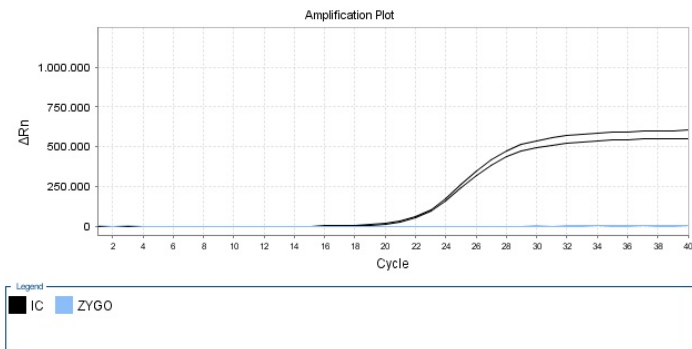
Zygosaccharomyces bailii

Ref: BIOPFS-0020

Positive Control –



Negative Control –



12. SPECIFICITY/INCLUSIVITY

- 100 % Exclusivity, determined using 33 strains of closely related organisms or occurring in the same habitat.
- 100 % Inclusivity, determined in 4 strains of *Zygosaccharomyces bailii*

13. SENSITIVITY

The REAL TIME DETECTION KIT *Zygosaccharomyces bailii* detects 10^2 - 10^3 cfu/45mL after sample concentration. This kit has a reaction sensitivity of 50 fg of target DNA.

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 synthesizes 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

Zygosaccharomyces bailii is a food spoilage yeast with high importance for wine, beverage, and food industry, because of its ability to grow in the presence of weak acid preservatives such as benzoic and sorbic acids. It has been identified as a cause of spoilage in alcoholic beverages such as wine and in foods such as mustard and soft drinks. *Z. bailii* is responsible for significant economic loss in the wine and food industry and its efficient detection is of the most importance. This detection is mostly performed using classical microbiological methods that rely on the growth of *Z. bailii* on selective media. This media is often exploiting its ability to grow in the presence of sorbate or other weak acids. The growth of *Z. bailii* on this media typically occurs between 4 and 5 days. Therefore, methods to shorten the time needed for the detection of *Z. bailii*, in wine, beverages, and food are desirable. 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 *Z. bailii* 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 *Zygosaccharomyces bailii* is intended for the rapid detection of *Z. bailii* 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:



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BPMR is certified ISO 9001:2015

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 -20°C 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	N° OF SAMPLES	
	1	10 (10 + 1)
qPCR reaction		
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	50°C	2 min.	No
	Step 2	95°C	5 min.	No
Amplification - 45 cycles	Step 1	95°C	30 sec.	No
	Step 2	56°C	30 sec.	Yes
	Step 3	72°C	30 sec.	No

TARGET	CHANNELS
Detection of <i>Z. bailii</i>	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:

	<i>Z. bailii</i> 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:

<i>Z. bailii</i> DETECTION FAM	IC DETECTION ROX	INTERPRETATION
Positive	Positive/Negative**	DNA detected, sample Positive for <i>Z. bailii</i>
Negative	Positive	No DNA detected, sample Negative for <i>Z. bailii</i>
Negative	Negative	Invalid Result*

*When both *Z. bailii* 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.