Xpert qDetect *Zygosaccharomyces bailii*

#GDK24.0100 (100 rxns)
(FOR RESEARCH ONLY)

Product: Wines, soft drinks, syrups, and dressings can potentially be spoiled by the presence of *Zygosaccharomyces bailii*. This yeast is extremely resistant to preservatives and tolerates high ethanol levels. In wine it can cause refermentation resulting in turbidity, sedimentation, and high levels of acetic acid and esters. *Z. bailii* is responsible for significant economic loss in the wine and food industry, making its detection of the utmost importance.

Detection of *Z. bailii* is usually carried out using traditional microbiological techniques based on incubating organisms on selective media, typically with long incubation time of 4-5 days. Therefore, methods to decrease detection time are extremely useful.

This qPCR Detection Kit provides a sensitive and reliable method for the detection of *Zygosaccharomyces bailii* based on qPCR using precisely designed specific primers and FAM-labeled Taqman® probe, requiring only a couple of hours. This immense time reduction allows winemakers to take appropriate action, if needed, much sooner. This kit is compatible with instruments equipped with FAM and ROX channels. The detection limit is approximately 50fg of *Z.bailii* DNA, or as little as 10^2-10^3 cells per 50ml of wine, with a specificity of 100%.

Applications: qPCR Detection of DNA from *Zygosaccharomyces bailii* (following DNA extraction from cells present in wine and other beverages (see: "prior to use")).

Contents: The qPCR Detection Kit (#GDK24.0100) for *Zygosaccharomyces bailii* contains sufficient reagent for 100 qPCR reactions.

Component	GDK24.0100
Zb Mix A	2x 840 µl
Zb Mix B	210 µl
Positive Control (Zb+)	70 µl
Negative Control (Zb-)	70 µl

Note: This product does not include reagents and other materials required for DNA extraction.

Samples: 2µl of DNA (previously purified from organisms present in 10-50ml of wine)

Properties: Fast, Easy and Reliable
Low limit of Detection
100% Specificity* (check our website for list of over 30 non-target microorganisms that can be found in the same environment and that were tested for possible interference).
Compatible with instruments equipped with **FAM** and **ROX** channels

Storage: -20ºC and protected from light for at least 1 year. Minimize repeated freeze/thawing, consider preparation of aliquots.

Prior to use:

This kit is meant for the detection of DNA from *Zygosaccharomyces bailii* present in total DNA previously purified from organisms present in wine. In order to obtain purified total DNA, one should concentrate cells, e.g. by passing 10-50ml through a filtration ramp using a filter with a 0.45µm pore size and subsequently extract DNA from cells on the membrane using an appropriate kit and manufacturer´s instructions. Unambiguous detection of *Zygosaccharomyces bailii* (and of the internal control) requires a suitable calibration of both FAM and ROX channel. Please refer to the manufacturer´s instruction of the qPCR cycler.

qDetect - Basic Protocol

1. **Mix for each qPCR reaction**:

Component	Volume
Zb Mix A	16 µl
Zb Mix B	2 µl

In order to minimize risk of contamination, reagent loss and improve pipetting accuracy, we recommend to prepare a mastermix for multiple samples (N), always including a negative control, and a positive control, by mixing all components (N+2), except template DNA (nor control DNA), dividing the mixture equally into each PCR tube (18 µl each), briefly spin tubes (or tap down) and then add 2 µl template DNA or control DNA directly in the mixture.

2. **Set-up qPCR cycling**:

Nº cycles	Temp	Time	Acquisition
1x	50ºC	2 min	No
1x	95ºC	5 min	No
45x	95ºC	30 sec	No
	56ºC	30 sec	Yes
	72ºC	30 sec	No

After an initial cycle of 2 min at 50ºC and 5 min at 95ºC (Enzyme activation and denaturation of template), cycle 45 times for 30 seconds at 95ºC, 30 seconds at 56ºC and 30 seconds at 72ºC. Acquire data for the detection of *Zygosaccharomyces bailii* on the **FAM** channel. Probe to detect specific amplification of the internal control, which is included in Zb Mix A and which is amplified simultaneously with the target DNA, should be detected in the **ROX** channel.

Results

Controls
In order to validate the assay, controls must have the following results. If the signal of one of the controls does not match, the whole experiment, including all samples, must be repeated.

Control	FAM channel	ROX channel
Negative Control	Ct=N/A	positive
Positive Control	positive	unimportant*

N/A = Not applicable (signal below threshold).
*unimportant: is expected to be positive, however, if negative but other controls match expected results, this makes no difference

Samples
For each sample, there are 4 possible outcomes, as summarized in the table below.

FAM channel	ROX channel	Result
positive	positive	positive
positive	Ct=N/A	positive
Ct=N/A	positive	negative
Ct=N/A	Ct=N/A	inhibition*

*) in case both *Zygosaccharomyces bailii* (FAM channel) and Internal Control (ROX channel) have signals below threshold, but all the controls resulted in signals as expected, the sample must be retested, as the qPCR reaction was inhibited. Inhibition often is the result of a too high DNA concentration and therefore it is recommended that retesting should be carried out with a 10-fold dilution of the original DNA sample.