dnature Wine & Beverage Spoilage qPCR Kits

v1.5 June 2022

BRETT100	**Brettanomyces bruxellenis qPCR Kit**	**100 tests**
ZYGOBAILII100	**Z. bailii qPCR Kit**	**100 tests**
YEAST100	**Total Yeast qPCR Kit**	**100 tests**
BACT100	**Total Bacteria qPCR kit**	**100 tests**
ZYGOROUXII100	**Z. rouxii qPCR Kit**	**100 tests**
LACACID100	**Lactic Acid Bacteria (Wine) qPCR Kit**	**100 tests**
LAC-BEER100	**Lactic Acid Bacteria (Beer) qPCR Kit**	**100 tests**
DIASTAT100	**Diastatic Yeast qPCR Kit**	**100 tests**

Introduction

A number of yeasts and bacteria are associated with spoilage in wine, beer and other beverages. Rapid detection of these organisms is important to mitigate their effects before spoilage occurs. In addition to specific spoilage organisms, tests to detect total bacteria and yeast are also available to detect unknown species that are not detected with specific protocols.

Tests

For ease of use, the multiple components are provided as a 20X concentration mix.
The mastermixes are provided at a 2X concentration. The intercalating dye mixes are colourless while the 'total bacteria' kit mastermix is blue.

Method

While most protocols use intercalating dye detection/melting curve, the 'total bacterial qPCR kit' uses hydrolysis probe methodology. This kit can be run together with the other kits, using the universal protocol below

DNA should be extracted from the wine, beer or other beverage by an appropriate method. Column-based kits are available from dnature for up to 2ml of sample.

Protocol (Per 10µl reaction)

Briefly vortex the 2X mastermix, 20X test-specific mix and pulse centrifuge prior to use

- Assemble a reaction cocktail comprising the following.
  Allow extra for pipetting (reaction vials contain 10% additional volume to account for pipetting additions)

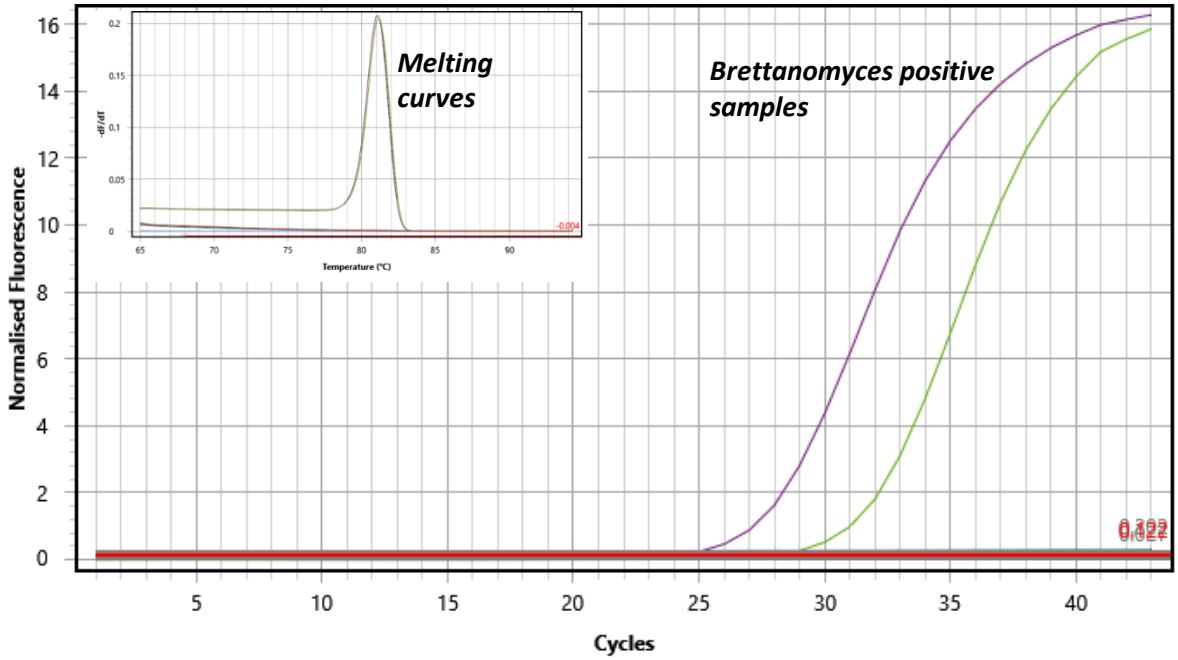
PCR-Grade Water	2.5 µL
20X test-specific mix	0.5 µL
2X Mastermix	5.0 µL

- Mix mastermix briefly by vortexing and pulse centrifugation before adding 8µl per well/tube, followed by 2µl DNA template (see notes)
- Seal tubes/plate and run as per instrument instructions

Suggested Run protocol (BMS Mic, for other instruments contact dnature)

Step 1	Activation	95°C 3 minutes
Step 2	Cycling	95°C 5 seconds] 60°C 20 seconds] x 43 cycles
Step 3	Melt	95°C 2 seconds 65°C 5 seconds Melt from 65°C to 95°C at 0.3°C/s

Analysis



Notes

- Assay cut-offs should be determined in own laboratory. In the dnature laboratory, reactions with a Cq <38 are deemed positive.
- Increased template volumes may be used by reducing water at corresponding volume.
- For rapid extraction methods (e.g. LysoFAST, dnature) the template volume should be reduced to 1 µL initially.

Storage

- Oligo Mix and mastermixes are stable for 6 months at 4°C.
- Unused tubes should be stored at -20°C with freeze/thaw steps minimised.
- For longer term storage (up to 1 year) they may be stored at -20°C.