

| Rev.: 002 | Issued March 2015 |
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| Read this package insert carefully before use | |

Oeno Yeast™

REF 05-6001

INTENDED USE

The OenoYeastTM reagent kit is intended to detect and encounter metabolic active microorganisms like yeast cells of the genus Dekkera/Brettanomyces in wine after termination of the alcoholic fermentation process.

Stained samples can be analysed on a flow cytometer.

KIT COMPONENTS

Packing contains reagents for 30 tests:

- 20 ml Solution A
- 0.4 ml Solution B
- 25 ml Count Check Beads Medium

INSTRUCTIONS

Sampling of wine samples from barrels during maturation (recommended procedure):

- draw sample barrel shortly above the deposit surface; use a specific disposable sampling device (syringe and 60 cm tube) or a vacuum device
- discharge the first 20 ml of sample and collect 125 ml of wine into a clean collection container
- clean sample equipment well before collecting the next sample
- make sure not to collect from the barrels deposit layer (visually control the collected sample is clear)

Sampling from bottles (recommended procedure):

- shake the bottle well before opening
- open bottle and collect 125 ml of wine sample into a clean collection container

Sample preparation:

Solution 1 and Solution 2 of the reagent kit should have room temperature before starting the preparation.

- add 400 µl of wine sample into a new sample tube (code 04-2000)
- add 400 µl of *Solution 1* and shake gently
- add 8 µl of Solution 2 and shake well or vortex
- incubate samples at room temperature for 10 minutes in the dark
- analyse wine sample on flow cytometer

Flow Cytometric Analysis:

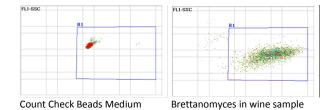
Instrument requirements and settings:

- Laser light source: 488 nm
- Detection of side scatter and green fluorescence emission light
- Trigger: green fluorescence detecting parameter
- Speed: 3 µl/sec

For instrument alignment and quality control, please refer to the IFU of your Flow Cytometer.

Analysis of wine samples:

- attach sample tube with *Count Check Beads Medium*
- optimize instrument set up
- set a gate around the beads in the dot plot of green fluorescence SSC
- run sample with *Cleaning Solution* (code: 04-4009)
- attach sample tube with the wine sample and start measurement
- cut off extensive background noise by increasing L-L threshold



For the count result of Brettanomyces consider the dilution factor of 2.02, caused by sample preparation.

Limitation of the procedure:

The lower detection limit of the method is 100 cells/ml. The lower detection limit can be decreased to 20 cells/ml after sample centrifugation (applicable only on clear wines). The upper limit of cell concentration is 10^6 cells/ml. Optimal concentration 10^4 to 10^5 cells/ml can be reached by sample dilution with *Solution 1*.

STORAGE AND STABILITY

| Storage: | 2- 8°C in the dark |
|-------------|----------------------------------|
| Shelf life: | Please refer to the expiry date, |
| | labeled on the bottle. |

DISPOSAL PROCEDURE

Disposal procedure should meet requirements of applicable local regulations.



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