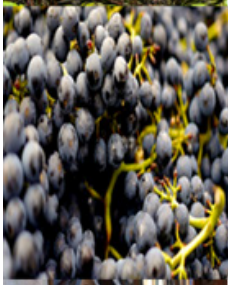


Micro biologique

Wine Spoilage Micro Test

Product Code: PM-05

Instructions For Use

Introduction

Microbes play a critical role in the wine making process but the presence of spoilage-causing microbes at any stage negatively affects the quality of wine. Microbial spoilage not only affects the flavor and shelf life of wine but also has a significant impact on the brand value. Therefore, simultaneous detection of yeasts (*Zygosaccharomyces, Brettanomyces*) and bacteria (*Lactobacillus, Pediococcus* and acetic acid bacteria) causing wine spoilage is of utmost importance to wineries. The Wine Spoilage Micro Test kit was developed to rapidly detect major spoilage microorganisms within two hours directly from a wine sample.

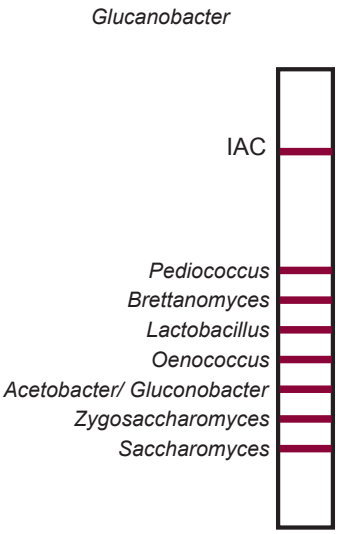
Intended Use

The test is designed to detect the presence of specific spoilage-causing yeasts and bacteria in wine. The test is intended for laboratory use only and should be performed by trained personnel.

Kit Storage and Stability

Store the PCR Buffer, Lysis Solution and Taq Beads at 2-8°C. Store the rest of the kit components at 2-25°C. The kit is stable until the expiration date indicated on the box if stored appropriately.

Principle of the Test



The Microbiologique Wine Spoilage Micro Test consists of two steps: **1)** concentration by centrifugation or enrichment using media that enhances growth of target spoilage organisms and **2)** detection of the target spoilage organisms by polymerase chain reaction (PCR). Primer probes unique to each spoilage organism are multiplexed and amplify the target genes. Application of the amplified product on a Lateral Flow (LF) strip results in labeling of the amplicons with gold nanoparticles and subsequent hybridization of the labeled amplicons to complementary regions on the strip. In addition, the LF strip also contains a control line (IAC – internal amplification control) to indicate the validity of both the PCR amplification and LF detection.

Performance Characteristics

Limit of Detection: 1 cell/mL

PCR operation and detection time: 2 hours

Cross-reactivity: Cross-reactivity was not observed in over 90 species of bacteria and fungi closely related to wine spoilage micro-organisms and commonly associated with food.

Kit Components (24 tests)

Lysis Solution (100 µL)
Taq Beads (3 strips of 8 tubes)
PCR Buffer (1.5 mL)
Lateral Flow Strips (24 strips)
Detection Reagent (4 mL)

Materials Required (but not Supplied)

Pipettors: 1 mL, 200 µL, 10 µL
Pipet tips with filter, sterile: 1 mL, 200 µL, 10 µL
Polypropylene conical tubes, sterile: 1.5 mL or 2 mL
0.2 mL thin-wall (PCR) tubes
50 mL polypropylene tube
Microcentrifuge capable of 10,000 *g*
Thermal cycler
Lateral Flow Strip Reader (Optional)
Vortex mixer

Required for Direct Detection Protocol:

Distilled water, sterile

Required for Enrichment Protocol:

Stomacher
7 oz Whirl Pak bag **OR** sterile containers with air tight cap
Wine Spoilage Enrichment Media Mix (Code B-1176, Microbologique)
Distilled water in appropriate-sized bottle for media preparation
Magnetic stirrer and stir bar
Autoclave
Incubator, 30 °C

Protocol

NOTE: Bring kit components to room temperature prior to PCR analysis.

Samples may be prepared for PCR analysis following the **Enrichment Protocol (Section A)** or **Direct Detection Protocol (Section B)** using a centrifugation step.

A. ENRICHMENT PROTOCOL

A1. Preparation of samples

1. Preparation of Enrichment Media

NOTE: Prepare sufficient amount of Enrichment Media for a **1:9** sample to Enrichment Media ratio.

a. Add the appropriate amount of Wine Spoilage Enrichment Media Mix in a clean glass bottle containing distilled water and a stir bar.

b. Mix the contents using a magnetic stirrer until dissolved.

c. Adjust the final volume with distilled water.

d. Autoclave at 121°C for 15 minuntes. Allow the media to cool to room temperature prior to use in the Enrichment step.

2. Enrichment step

a. Aseptically mix the autoclaved Enrichment Media from Step **A1.1** with the wine sample at a **1:9** ratio.

For example: Transfer 100 mL of wine sample to a sterile container and aseptically add 900 mL of Enrichment Media.

b. Secure the container using an air tight cap, mix well by inverting the container or stomaching the WhirlPak bag for at least 30 seconds and incubate at 30°C for 24-48 hours.

NOTE: Incubate for 48 hours to enrich for yeasts. However some strains of *Brettanomyces* can take more than three days for optimal growth; thus longer incubation period may be required.

c. After the incubation period, proceed to sample lysis step (**Section A2. PCR analysis)**.

A2. PCR analysis

1. Sample lysis

a. Aliquot 1 mL of the prepared enrichment sample in a sterile 2 mL polypropylene conical tube.

b. Centrifuge the sample at 10,000 *g* for 5 minutes.

c. After centrifugation, carerfully remove the supernatant and avoid disturbing the pellet.

d. Obtain the PCR Buffer and vortex the tube to ensure a homogenous solution prior to use. **NOTE:** Light precipitation may be observed but this does not affect the functionality of the PCR Buffer.

e. Re-suspend the pellet from Step **A2.1.c** using 50 µL of the PCR Buffer and mix well.

NOTE: The volume of **PCR Buffer** may be increased to dissolve a larger pellet but **should not exceed 150 µL**.

f. Transfer 50 µL of the mixture into a 0.2 mL PCR tube then add 2 µL of Lysis Solution to the mixture. Mix or vortex the tube briefly.

g. Incubate the tube in a thermal cycler using the pre-PCR Lysis conditions (Inc 37) below: **1 cycle:** 37°C, 10 minutes
99°C , 5 minutes
4°C (∞)

h. After the cycle, proceed to the PCR amplification steps.

2. PCR amplification

a. Transfer 25 µL of the lysed sample to the Taq Bead tube.

b. Briefly vortex the tube, pulse-centrifuge and transfer to a thermal cycler. Perform amplification following the PCR cycling conditions below:
1 cycle: 95°C, 4 minutes
35 cycles: 95°C,10 seconds **/** 64°C, 30 seconds **/** 72°C,10 seconds
Final: 4°C, ∞

c. Once the PCR amplification is complete, proceed to the amplicon detection steps using an LF strip.

3. Amplicon detection using LF strips

a. Briefly vortex the PCR tube containing the amplicon (PCR product).

b. Take 10 µL of amplicon and mix well with 120 µL of Detection Reagent in a sterile 1.5 mL polypropylene conical tube. Apply 120 µL of the mixture onto the LF Strip sample pad.

c. Read results visually at 15-20 min by aligning your LF Strip next to the Reference Lateral Flow Strip on **Page 12** or by using an LF Strip reader.

B. DIRECT DETECTION PROTOCOL (Centrifugation)

B1. Preparation of samples

1. Mini Protocol (Medium sensitvity - 10 cells/mL)

a. Mix the sample well by gentle swirling to avoid frothing.

b. Aliquot 1.8 mL of sample into a sterile 2 mL tube and cap tightly.

c. Place capped tubes in a microcentrifuge and ensure that they are balanced.

d. Centrifuge at 10,000 *g* for 5 minutes.

e. Using a 1 mL pipettor and a fresh pipet tip, carefully remove the supernatant and avoid disturbing the pellet.

f. Obtain the PCR Buffer and vortex the tube to ensure a homogenous solution prior to use.

NOTE: Light precipitation may be observed but this does not affect the functionality of the PCR Buffer.

g. For large pellet

i. Using a 200 µL pipettor, re-suspend the pellet using 50 µL of **sterile distilled water**.
NOTE: The volume of water may be increased to dissolve a larger pellet but **should not exceed 100 µL.**

ii. Mix well by vortexing.

iii. Prepare the Lysis Buffer by transfering 50 µL of PCR Buffer into a 0.2 mL PCR tube, then add 2 µL of Lysis Solution to the buffer.

iv. Transfer 2 µL of cell suspension from Step **B1.1.g.i** into the 0.2 mL PCR tube containing the Lysis Buffer.

v. Vortex briefly and proceed to the sample lysis procedure.

h. For very little to no visible pellet (as for most bottled or filtered wine).

i. Add 50 µL of **PCR Buffer** (instead of distilled water) to the tube and mix well by vortexing.

ii. Transfer the entire volume from Step **B1.1.h.i** into an empty 0.2 mL PCR tube, then add 2 µL of Lysis Solution to the buffer.

iii. Vortex briefly and proceed to the sample lysis procedure.

2. Maxi Protocol (High sensitvity - 1 cell/mL)

a. Mix the sample well by gentle swirling to avoid frothing.

b. Aliquot 45 mL of sample into a sterile 50 mL tube and centrifuge at 6,000 *g* for 5 minutes.

c. Carefully remove the supernatant (without disturbing the pellet) and re-suspend the pellet using 1 mL of sterile distilled water.

d. Transfer the contents into a 2 mL tube and centrifuge at 10,000 *g* for 5 minutes.

e. Using a 1 mL pipettor and a fresh pipet tip, carefully remove the supernatant to avoid disturbing the pellet.

f. Obtain the PCR Buffer and vortex the tube to ensure a homogenous solution prior to use.
NOTE: Light precipitation may be observed but this does not affect the functionality of the PCR Buffer.

g. For large pellet

i. Using a 200 µL pipettor, re-suspend the pellet using 50 µL of **sterile distilled water**.
NOTE: The volume of water may be increased to dissolve a larger pellet but **should not exceed 100 µL.**

ii. Mix well by vortexing.

iii. Prepare the Lysis Buffer by transfering 50 µL of PCR Buffer into a 0.2 mL PCR tube, then add 2 µL of Lysis Solution to the buffer.

iv. Transfer 2 µL of cell suspension from Step **B1.2.g.i** into the 0.2 mL PCR tube containing the Lysis Buffer.

v. Vortex briefly and proceed to the sample lysis procedure.

h. For very little to no visible pellet (as for most bottled or filtered wine).

i. Add 50 µL of **PCR Buffer** (instead of distilled water) to the tube and mix well by vortexing.

ii. Transfer the entire volume from Step **B1.2.h.i** into an empty 0.2 mL PCR tube, then add 2 µL of Lysis Solution to the buffer.

iii. Vortex briefly and proceed to the sample lysis procedure.

B2. PCR analysis

1. Sample lysis

a. Incubate the sample tube in a thermal cycler using the pre-PCR Lysis conditions (Inc 37) below:

1 cycle: 37°C, 10 minutes
99°C , 5 minutes
4°C (∞)

b. After the sample lysis cycle, proceed to the PCR amplification steps.

2. PCR amplification

a. Transfer 25 µL of lysed sample to the Taq Bead tube.

b. Briefly vortex the tube, pulse-centrifuge and transfer to a thermal cycler. Perform amplification following the PCR cycling conditions below:

1 cycle: 95°C, 4 minutes
35 cycles: 95°C,10 seconds **/** 64°C, 30 seconds **/** 72°C,10 seconds
Final: 4°C, ∞

c. Once the PCR amplification is complete, proceed to the amplicon detection steps using an LF strip.

3. Amplicon detection using LF strips

a. Briefly vortex the PCR tube containing the amplicon (PCR product).

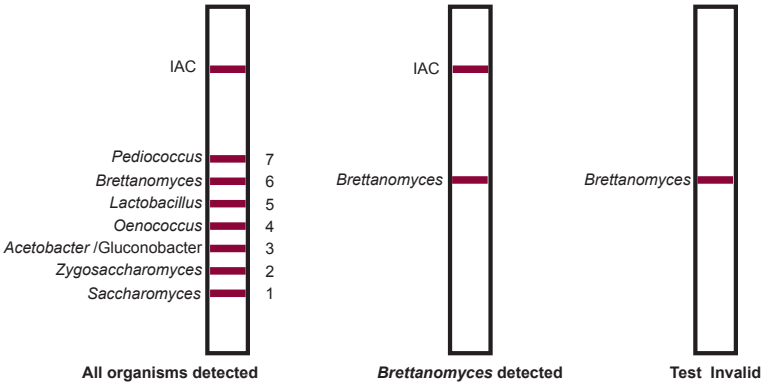
b. Take 10 µL of amplicon and mix with 120 µL of Detection Reagent in a sterile 1.5 mL polypropylene conical tube. Apply 120 µL of the mixture onto the LF Strip sample pad.

c. Read results visually at 15-20 min by aligning your LF Strip next to the Reference Lateral Flow Strip on **Page 12** or by using an LF Strip reader.

Interpretation of the Test

The test is valid and further interpretations can be made only if the top line representing the IAC (internal amplification control) appears on the LF strip. As graphically shown below*, a series of lines may appear depending on the type of spoilage organism(s) present in the sample.

For example, if Line 6 appears as well as the IAC , then the sample can be reported for the presence of *Brettanomyces*. However, if only Line 6 appears with no IAC line then the test is invalid and needs to be repeated.



**** Not drawn to scale***

Limitations

As with all assays requiring PCR amplification, the presence of inhibitors in the sample should be considered in the interpretation of test results as this may lead to inaccurate or invalid results. The test kit has been validated for common types of wine – red (merlot) and white (chardonnay) wines. Additional validation is recommended to verify suitability for use with other types of wine. Questions regarding sample suitability as well as recommended LF strip reader for use in recording test results may be addressed to customer support.

Precautions

For Laboratory use only. The test should be performed by trained personnel. The kit may be used as part of quality control testing by vintners for their products. Operation of the test should be performed using Good Laboratory Practices and using personal protective equipment including gloves, lab coat and safety glasses. Strict adherence to the assay protocol is mandatory to ensure proper operation. Do not mix kit components with ones from kit with different lots. To limit contamination, avoid creating aerosols or aspirating when pipetting. SDS information can be obtained from your distributor or by emailing: tech@microbiologique.com.

Customer Support

For additional information on using this test kit, please contact:

1.888.998.4115 (USA & Canada)

+1.206.525.0412 (International)

Email: tech@microbiologique.com

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Manufactured by Microbiologique, Inc.
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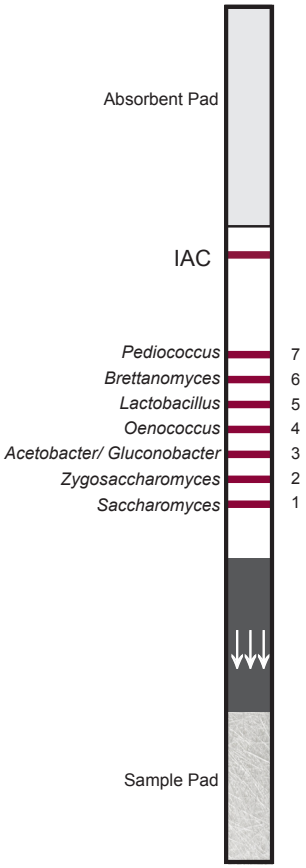
USA

Wine Spoilage Micro Test

Reference Strip

Procedure: Align the test strip with reference IAC line in the diagram below.



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