Micro biologique

Beer Spoilage Micro Test

Product Code: PM-02

Instructions For Use

Introduction

Beer spoilage caused by wild yeasts and bacteria is a major concern to both commercial and home brewers. Microbial spoilage not only affects the shelf life of beer but also has a significant impact on the brand value. Therefore, simultaneous detection of wild yeasts (*Saccharomyces diastaticus, Brettanomyces*) and bacteria (hops-resistant Lactic acid bacteria, *Megasphaera* and *Pectinatus*) causing beer spoilage is of utmost importance to brewers. The Beer Spoilage Micro Test kit was developed to rapidly detect major spoilage microorganisms within 2 hr directly from a spoiled beer sample or following a 24-48 hr enrichment step (recommended for clear beer).

Intended Use

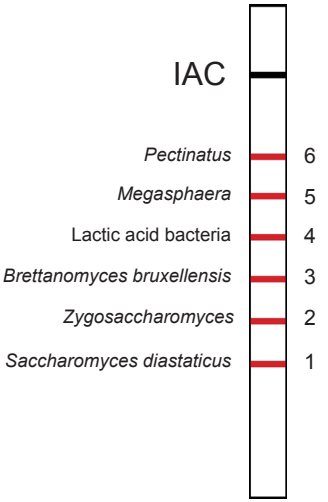
This kit is intended for the detection of specific spoilage-causing yeasts and bacteria in beer. The test is intended for laboratory use only and should be performed by trained personnel.

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.

IFU-PM-02 REV 5.0, 20 Sep 2017

Principle of the Test



The Microbiologique Beer Spoilage Micro Test consists of two steps: a) concentration by centrifugation or enrichment using media that enhances growth of target spoilage organisms and b) detection of the target spoilage organisms by polymerase chain reaction (PCR). To operate this kit, the sample is first subjected to a concentration or an enrichment step followed by amplification of specific genes in the target spoilage organisms. 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 hybridization of 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 - 10 cells/mL

Sample enrichment (Optional): 24 - 48 hr

PCR operation and detection: 2 hr

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

Kit Components (24 tests)

Lysis Solution (100 µL)
Taq Bead (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
Sterile 1.5-mL or 2-mL polypropylene conical tubes
0.2 mL thin-wall (PCR) tubes
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 and 7 oz Whirl Pak bag **OR** 50 mL conical tube
Beer Spoilage Enrichment Media Mix (Code B-1162, 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
(Recommended for clear beer samples)

A1. Preparation of samples

1. Preparation of Enrichment Media

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

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

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

c. Autoclave at 121°C for 15 min. Allow the media to cool to room temperature prior to use.

2. Enrichment step

a. Aseptically mix Enrichment Media with the beer sample at 1:1 ratio by carrying out either of the following procedures:

- Remove 150 mL of beer from the 300 mL beer bottle and aseptically replace with 150 mL of Enrichment Media.

OR

- Transfer 25 mL of beer sample to a 50 mL conical tube or WhirlPak bag and aseptically add 25 mL of Enrichment Media.

b. Close the containers securely, mix well by inverting the tube or stomaching the WhirlPak bag for at least 30 seconds and incubate at 30°C for 24 hr.

NOTE: Incubate for **48 hr** to enrich for **yeasts** and ***Pediococcus damnosus*** (one of the Lactic acid bacteria targets).

c. After the incubation period, proceed to sample lysis.

A2. PCR analysis

1. Sample lysis

a. Aliquot 1 mL of the prepared enrichment sample in a sterile 2 mL polypropylene conical tube and centrifuge at 10,000 *g* for 5 min.

b. Obtain the PCR Buffer and gently swirl the tube to ensure a homogenous solution prior to use. Light precipitation may be observed but this does not affect the functionality of the PCR Buffer.

c. Carerfully pipet out the supernatant of the centrifugation from step **1.a** then re-suspend the pellet using 50 µL of the PCR Buffer. Mix well. **NOTE:** The volume of **PCR Buffer** may be increased to dissolve a larger pellet **but should not exceed 150 µL**.

d. 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.

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

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

f. After the 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 min
35 cycles: 95°C, 10 sec, 64°C, 30 sec, 72°C, 10 sec
Final: 4°C, ∞

c. Once the PCR amplification is complete, proceed to the amplicon detection steps on a DNA LF strip.

3. Amplicon detection using DNA LF strip

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 Lateral Flow Strip sample pad.

c. Read results visually at 15-20 min by aligning your Lateral Flow Strip next to the Reference Lateral Flow Strip on the final page of this IFU or using an LF Strip reader.

B. DIRECT DETECTION PROTOCOL (Centrifugation)
(Recommended for grossly contaminated samples)

B1. Preparation of samples

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

b. Using a 1 mL pipettor, transfer 1 mL of the sample into a sterile 1.5 or 2 mL polypropylene conical 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 min.

e. Using a 1 mL pipettor and a fresh pipet tip, pipet out supernatant with care to avoid disturbing the pellet.

f. Using a 200 µL pipettor, re-suspend pellet in 50 µL of sterile distilled water. Mix well by vortexing.

NOTE: The volume of **distilled water** may be increased to dissolve a larger pellet **but should not exceed 100 µL.**

g. 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.

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

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

B2. PCR analysis

1. Sample lysis

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

1 cycle: 37°C, 10 min
99°C , 5 min
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 min
35 cycles: 95°C, 10 sec, 64°C, 30 sec, 72°C, 10 sec
Final: 4°C, ∞

c. Once the PCR amplification is complete, proceed to the amplicon detection steps on a DNA LF strip.

3. Amplicon detection using DNA LF strip

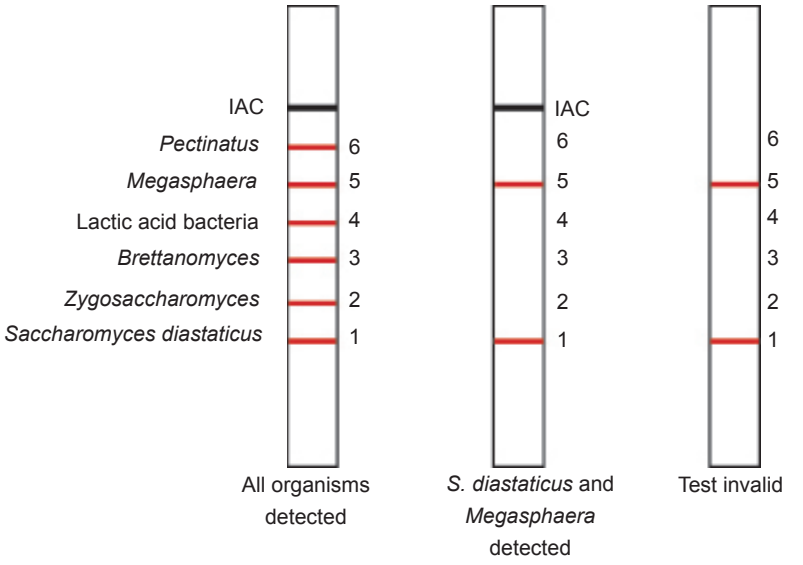
a. Briefly vortex the PCR tube containing the amplicon (PCR product).

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 Lateral Flow Strip sample pad.

c. Read results visually at 15-20 min by aligning your Lateral Flow Strip next to the Reference Lateral Flow Strip on the final page of this IFU or using an LF Strip reader.

Interpretation of the Test

The test is valid and further interpretations are 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 1 and Line 5 appear (+ IAC), then the sample can be reported for the presence of *Saccharomyces diastaticus* and *Megasphaera.* However, if only Line 1 and Line 5 appear, then the test is invalid and needs to be repeated.



**** Not drawn to scale***

Reference Strip

Align strip with IAC line.

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 beer such as ale and lager, thus validation is recommended to verify suitability for use with other types of beer. Questions regarding sample suitablility as well as questions concerning the LF strip reader that is recommended 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 and may be used as part of quality control testing of brewers 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 other kits or kit lot numbers. 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

Disclaimer

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