1. PRODUCT DESCRIPTION

GENIUL_ 4900020000

Wine vPCR kit

What this product does

Wine vPCR kit has been developed to improve the detection of spoilage microorganism during the wine production, by viability PCR.

After the treatment of spoilage microorganism suspension with this kit, only DNA from living microorganism will be detected by molecular procedures such as PCR.

This kit is a sample pretreatment previous to DNA extraction and detection, it doesn't include PCR reagents and not is a detection kit by itself.

This kit comprises the use of two Buffers, Reaction Buffer (R) and Dilution Buffer (D).

R Buffer is an acidic-organic base Buffer designed as a holding medium for maintaining the viability of wine spoilage microorganisms and compatible with viability dyes. D Buffer is a mineral base Buffer compatible with lytic reagents used in DNA purification procedures.

The combination of the Buffers along our PEMAX reagent is a technology developed by GenIUL.

http://www.sciencedirect.com/science/article/pii/S0890850815000304

This product ensures the optimal sample preparation and the maximum signal PCR reduction of dead cells in wine spoilage microorganisms (bacteria and yeasts), such as *Brettanomyces* spp.

Form: Liquid

Storage instructions: All components (PEMAX Reagent, Reaction Buffer (R) and Dilution Buffer (D)) must be stored in a lab fridge (2-8 ºC).

Intended use: For Research Use Only.

Contents

This Kit contains reagents for 75 samples.

Reaction Buffer for wine samples (R), 60 mL	3 units
Dilution Buffer for wine samples (D), 60 mL	1 unit
PEMAX Reagent 2 vials of 0,5 mg. (Cat. No. 4900013000)	2 units

Storage & Shelf life

Upon receipt, store all components (PEMAX Reagent, Reaction Buffer (R) and Dilution Buffer (D) in a lab fridge. Protect from direct light.

- This product will be stable at least for 6 months from date of manufacture.
- For R and D Buffer, temperatures below 6 ºC (44 degrees F) may cause the content precipitate. Previous to use, allow tempering and mix up to complete dissolution.
- Reaction Buffer (R) contains organic matter, it may cause microbial growth if the contamination is not prevented. Once opened, preferably use the Buffer within the next two weeks. Discard the Buffer if turbidity is detected.

Additional equipment required but not provided

- GenIUL Photo-activation system: PhAST Blue (Cat. No. 9000700)
- Isothermal system for micro-tubes incubation: Dark box system (Cat. No. 90001200)
- Reaction tubes (Cat. No. 4900019000)
- D-Bag system: D-Bag Holder (Cat. No. 900099645)
- Decontaminating Bag (Cat. No. 4900009500)
- Laboratory centrifuge and pipettes

Microbiological state

Sterile product (s).

Specimen & Reagent preparation

Refer to procedure. See section 2: Operating procedure.

Applicability statement

The amount of reagent contained is sufficient to neutralize the DNA of at least 1.10^5 dead cells or cells with damaged membrane.

This product has been formulated according to complains the range of reagents concentration needed for 500 µL of reaction volumes. Working with low volumes will increase reagents concentration, however some of them can become toxic for some microorganism.

This product has been successfully tested with wine spoilage microorganisms. The ingredients contained in this mix can be toxic for other microorganism.

General Rules

Please, carefully read the MSDS of this product.

This product is sold for research purposes. It is not intended for food, drug, household, agricultural or cosmetic use. Its use must be supervised by a technically qualified, individual experienced, in handling potentially hazardous chemicals.

Follow the general procedures of a molecular biology laboratory, especially those aimed at preventing cross-contamination. It is advisable to use micro-pipette tips with filter and the use of gloves and personal protection. The use of biological safety cabinets is recommended if you believe that your sample handling system can generate aerosols.

Users should make independent decisions regarding completeness of the information based on all sources available.

GenIUL shall not be held liable for any damage resulting from handling or contact with the above product.

2. OPERATING PROCEDURE

2.1. Important points before starting

When working with chemicals, always wear a suitable lab coat and disposable gloves. For more information, consult the appropriate MSDS, available from the product supplier.
Follow the general procedures of a molecular biology laboratory, especially those aimed at preventing cross-contamination. It is advisable to use micro-pipette tips with filter and the use of personal protection.

The use of biological safety cabinets is recommended if you believe that your sample handling system can generate aerosols. If working with infectious pathogens, the viability qPCR protocol and subsequent sample analysis (qPCR) must be carried out in a suitable laboratory area, designated for working with infectious pathogens.

2.2. Material Checklist

- PhAST Blue device and Dark Box system
- Reaction tubes
- Reaction Buffer (R)
- PEMAX Reagent
- Dilution Buffer (D)

2.3. Overview of the viability qPCR workflow

The general setup of the detection of viable spoilage microorganisms in wine using GenIUL's viability qPCR technology is illustrated in our Doc. Code 450000090. The workflow consists of 4 main segments, which include concentration of the wine sample (step 1), sample treatment with vPCR Reagent (steps 2-3), photo-activation of reagents (step 4), DNA purification (step 5) (reagents not provided) and qPCR (step 6) (reagents not provided).

In the following, a short description of each step will further describe and explain the different workflow segments.

- **1**: Sample concentration and washing.

The starting material is a suspension containing a mixture of live and dead cells from production samples or finished product. The quality of the results is highly influenced by the sample quality; for this reason is very important remove the PCR inhibitors or background materials as possible.

Starting sample:

- For finished product, concentrate 20 mL by centrifugation at 8000xg during 5 min or 3500xg during 30 min at <10ºC. Carefully remove the supernatant up to obtain a 1,7 mL of concentrated sample. If is necessary, add Reaction Buffer. Transfer the sample to a reaction tube, and proceed as stated below for production samples.

- For production samples (use reaction tube for sampling), concentrate 1.7 mL by centrifugation at 8000xg during 5 min., remove the supernatant and re-suspend with 1 mL of Reaction Buffer. Centrifuge the sample at 100xg during 2 min and collect the supernatant in a new reaction tube. Concentrate the sample by centrifugation at 8000xg during 5 min, remove the supernatant and re-suspend with 1 mL of Reaction Buffer. Repeat this last step one time more up to obtain a clean pellet this time re-suspend the pellet in 0,475 mL of Reaction Buffer.

- **2:** Reagent incubation.

Once re-suspended the pelleted sample in 0,475 mL of Reaction Buffer, carefully add 25 µL of PEMAX Reagent, previously dissolved the vial in 500 µl of sterile be distilled or PCR grade water (not provided), minimizing light exposure. The PEMAX Reagent is sensitive to light. Therefore is highly necessary to avoid light exposure at this stage, for this reason the use of our Dark Box system is recommended. Incubate on darkness, at room temperature (20-25ºC) during at least 20 min.

- **3**: Reagent activation.

Remove the tube holder from the Dark Box. Spin down the sample, homogenise the cells with the use of the micropipette, and transfer all samples to a new tube. Insert the tube into the PhAST Blue device. Perform a light treatment of 15 min. at 100% power, 10 min on darkness, and 15 min light at 100% power. Once the sample has been photo-activated, repeat the previous steps to transfer the sample to a new tube.

- **4**: Reaction Buffer removal.

Your sample is ready for analysis with a qPCR procedure, however the current pH of the sample not is compatible with most of enzymatic digestions used in DNA purification procedures. Additionally, the high levels of Phenantridinium can become PCR inhibitors. For this reason is recommended to perform an additional centrifugation step, at least 8000xg during 5 min, remove the supernatant and re-suspend the pellet with 200 µL of Dilution Buffer (D).

> **NOTE**: If the next step is the DNA extraction procedure with VINEO™ Extract DNA kit of Bio-Rad, the pellet resuspension step with Dilution Buffer (D) is not necessary to perform, at this stage, follow step protocol "2º) Cell lysis" indicated in the manufacturer's instructions (Doc. Code 881182 - 2015/09).

Two steps of boiling (95ºC, 10 min) and freezing (-20ºC, 10 min) samples prior to the DNA purification protocol is recommended to destroy the cells and thus facilitate DNA extraction, especially for yeast cells.

Residues containing non photo-reactive Phenantridinium can retain certain levels of dyes with reversible DNA interaction. For this reason, as happens with other DNA staining reagents, these residues should be handled with care and managed according good laboratory practices and national regulations. For this purpose, we recommend to fix these residues using our Decontaminating Bag system.

2.4. Procedure

Sample concentration

Steep	Action
1	Before collection, shake the sample container and decant 10 minutes.
2	Concentrate 1,7 mL (production samples) or 20 mL (finished product) by centrifugation (8000xg, 5 min or 3500xg, 30 min, <10ºC; respectively) up to 1,7 mL.

Sample washing

3	Eliminate the supernatant and re-suspend the pellet with 1,7 mL of Reaction Buffer (R).
4	Homogenize the sample with the aid of a vortex or micro-pipette.
5	Centrifuge the sample at 100xg during 2 min and collect the supernatant in a new reaction tube.
6	Concentrate the wine sample by centrifugation (5 min, 8000xg) and re-suspend the pellet with 475 µl of Reaction Buffer (R).

Reagent incubation

7	Add 25 µl of PEMAX Reagent and homogenize.
8	Immediately transfer the Reaction tube to the Dark Box for incubation on darkness during at least 20 min at room temperature (20-25ºC).
9	Spin down the tube, homogenize the cells with the use of the micropipette, and transfer all samples to a new tube.

Reagent photo activation

10	Place the tube holder into the PhAST Blue system.
11	Perform a light treatment of 15 min at 100% power, 10 min on darkness, 10 min on darkness, and 15 min light at 100% power.

Removing reagents from the mix and prepare the sample for DNA purification

12	Spin down the tube, homogenize the cells with the use of the micropipette, and transfer all samples to a new tube.
13	Concentrate the sample by centrifugation (5 min, 8000xg), eliminate the supernatant.
14 *	Re-suspend the pellet with 200 µl of Dilution Buffer (D).
15	Optional: Perform two steps of boiling (95ºC, 10 min) and freezing (-20ºC, 10 min) cells prior to DNA purification protocol.

*** NOTE**: If the next step is the DNA extraction procedure with VINEO™ Extract DNA kit of Bio-Rad, the pellet resuspension step with Dilution Buffer is not necessary to perform, at this stage, follow step protocol "2º) Cell lysis" indicated in the manufacturer's instructions (Doc. Code 881182 - 2015/09).

3. FREQUENTLY ASKED QUESTIONS

Download from our web page the current version of our FAQs in vPCR (Doc. Code 450000025) or contact with us (geniul@geniul.com), your doubts are very important for us and we want to help you.

4. WARRANTY AND DISCLAIMER OF LIABILITY

GenIUL warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

(1) The product is used according to the guidelines and instructions set.

(2) GenIUL does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by GenIUL; defects caused by misuse or use contrary to the Instructions supplied, or if the product is contaminated by improper handling or storage.

(3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the manufacturing date. There are no other warranties that extend beyond those described in this document.

(4) GenIUL does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of GenIUL.

(5) GenIUL does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, and expenses for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death.

(6) GenIUL reserves the right to replace or allow credit for any modules returned under this warranty.

5. OTHER INFORMATION

This product has been released for evaluation and research purposes.

Some applications, in which Phenantridinium can be used, may be covered by patents issued and applicable in the United States, Japan and certain other countries. The use of this product not contains any external license or right for their commercial use.

The use of this product is covered by Licenses, patents or patent pending request belonging to GenIUL. The customers that received this product can use it for research and evaluation purposes without infringing intellectual property rights.

It is not intended for food, drug, household, agricultural or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. Users should make independent decisions regarding completeness of the information based on all sources available. GenIUL shall not be held liable for any damage resulting from handling or contact with the above product.

6. CONTACT AND SUPPORT

If you have questions or experience problems with this or any other product of GenIUL, please contact our technical support staff (see details on www.geniul.com). Our scientists are committed to provide assistance quickly and effectively. We also would like you to contact us if you have suggestions to improve our product performance or the use of our products in new forms or applications.

Instruments Útils de Laboratori GenIUL, S.L.
Edifici GAIA - Parc UPC
Rambla de Sant Nebridi 22
08222 Terrassa (Barcelona)
Spain
Tel: +34 93 619 03 12 / Fax: +34 93 619 74 91
geniul@geniul.com