## 1. PRODUCT DESCRIPTION

**GENIUL_4900021000**

***Bifidobacterium* spp. qPCR detection Kit**

### Purpose of this product

This qPCR detection Kit has been developed in order to meet the specifications needed for a vPCR workflow.

It has been designed for use with standard cycling mode on standard and fast qPCR platforms. This detection Kit includes a novel reference proprietary dye blend that overcomes the current limitations of some qPCR dyes such as partial PCR inhibition or preference for some base pairs. This fact is important in vPCR application because it often requires large amplicons, with variable GC% regions.

It also can be used on all real-time PCR instruments requiring normalization with ROX, without modifications or adjustments to the specific instrument or protocol. ROX reference dye not provided within the kit.

### Intended use

This kit is intended for quality control purposes or research use. The product is not intended for the diagnosis, prevention, or treatment of a disease.

### Contents

*Bifidobacterium* spp. qPCR detection Kit contains all components necessary to perform the analysis; reagents, enzymes, optimized mixture of PCR and a dye blend comprised for two DNA-intercalating dyes that shows many features that make it an alternative to current dyes for qPCR. The absorption wavelength of dyes is 488 nm and their emission wavelength 509 nm, being compatible with all common real-time PCR cyclers, simply select the standard settings for EVAGreen®, SYBR® Green I or FAM. Furthermore, each kit includes positive DNA control (*Bifidobacterium longum* CECT 4551).This kit is designed to perform 50 reactions (25 µl each). The delivery includes the following items:

**Table 1.** *Bifidobacterium* spp. qPCR detection Kit contents

| Reagents (50 reactions) | Color code | Contents |
|---|---|---|
| 5x Reagent mix (*) | Green Cap | 275 µl |
| qPCR Assay (**) | Orange Cap | 1 x 50 reactions |
| Standard DNA (Positive control of amplification and Standard curve) | Yellow Cap | 1 x 50 reactions |
| RNase-Free water | White Cap | 1500 µl |

(*) Reagent mix: **GenIUL** proprietary dye blend and **HOT FIREPol® qPCR Mix Plus** (contains HOT FIREPol® DNA Polymerase, DNA-intercalating dyes, qPCR buffer, and dNTP mix -dATP, dCTP, dGTP, dTTP-)

(**) Contains target-specific primers

EVAGreen® is a trademark of Biotium, SYBR® Green is a trademark of Molecular Probes and Firepol ® is a trademark of Solis Biodyne.

## Additional Equipment Required

- Nucleic acid isolation kit
- Pipets (adjustable)
- Sterile pipet tips with filters
- Thermal cycler for Real Time PCR
- PCR consumables for the thermal cycler to be used
- Tube rack
- Microcentrifuge
- Vortex

## Storage & shelf life

Routine storage: -20ºC
This qPCR detection Kit is shipped at room temperature. Shipping storage at room temperature has no detrimental effects on the quality of 5x HOT FIREPol® qPCR Mastermix due to this qPCR detection Kit incorporates the stability TAG technology developed by Solis BioDyne.
It should be stored at -20ºC upon receipt, in a constant-temperature freezer and protected from light. When stored under these conditions and handled correctly, assay performance remains unaffected until the date of expiration printed on the quality control label inside the kit box or envelope.
Reconstituted reagents of this Kit should be dispensed into aliquots to avoid more than 5 freeze-thaw cycles, and stored at -20ºC for long-term storage.

## Microbiological state

Sterile product(s).

## Specimen & reagent preparation

Refer to Procedure. See section 2: Operating procedure

## General Rules

Please, read carefully MSDS of this product before use.
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.
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. Things to do before starting

- Reconstitute the dried Standard DNA (Yellow cap tube).

  Add 70 µl of RNase-Free water (white cap tube) to the vial and mix by pipetting up and down 5 times or vortexing. Centrifuge briefly. Use this solution for the preparation of defined Standard DNA dilutions. Assign the copy numbers given in Table 2. Use Standard 2 DNA dilution as Positive PCR control. Make appropriate aliquots to avoid more than 5 freeze-thaw cycles, and store the aliquots at -20ºC for long-term storage.

- Reconstitute the qPCR Assay (orange cap tube).

  Add the entire volume (275 µl) of the 5x HOT FIREPol® qPCR Mastermix (Green cap tube) to the qPCR Assay vial (Orange cap tube) and mix by pipetting up and down 5 times or vortexing. Do a short spin. If all the reagent won't be used, consider making appropriate aliquots to avoid more than 5 freeze-thaw cycles, and store the aliquots at -20ºC for long-term storage.

- Before each use, ensure that all reagents need to be thawed completely and do a short spin.

- Table 2 indicates how to make the standard DNA dilution for qPCR procedures. If you don't need to work with a complete calibration standard at least include one positive control in each run (standard 1).

**Table 2.** Preparation of the Standard DNA dilution series

| Standard DNA | Dilutions | Copy number per PCR reaction (5µL) |
|---|---|---|
| 1 | Standard DNA dissolved in 70 µl RNase-Free water | 2,000,000 |
| 2 | 5 µl Standard 1 + 45 µl RNase-Free water | 200,000 |
| 3 | 5 µl Standard 2 + 45 µl RNase-Free water | 20,000 |
| 4 | 5 µl Standard 3 + 45 µl RNase-Free water | 2,000 |
| 5 | 5 µl Standard 4 + 45 µl RNase-Free water | 200 |
| 6 | 5 µl Standard 5 + 45 µl RNase-Free water | 20 |

## 2.2. Procedure

1. Set up the sample and control reactions according to Table 3. If the addition of ROX is needed, use the recommended ROX concentrations according to Table 4. Keep all samples and reaction tubes on ice, or equivalent system, during setup. The good laboratory practice needs the inclusion of different controls; please don't forget to include al least the Positive control (Standard DNA) and Negative control (RNase-Free water).
2. Close the PCR tubes or strips and place them in the reaction chamber of the thermal cycler, securing them according to the instrument manual.
3. Program the thermal cycler. Use the recommended cycling protocol to Table 5. For data analysis, select green (FAM) channel.
4. Start the PCR run.
5. Proceed with the analysis results.

**Table 3.** Recommended volumes for single qPCR reaction mix. For each run, multiply by the total samples and 1,1 for ensuring no lack of reagents.

| **Component (x1 reaction)** | **Volume** |
|---|---|
| 5x HOT FIREPol® qPCR Mastermix with qPCR Assay primers | 5 µl |
| ROX (Optional but not provided) | See Table 4 |
| RNase-Free water | Up to 20 µl |
| **Reaction volume** | 20 µl |
| Sample DNA /Standard DNA/ RNase-Free water | 5 µl |
| **Total reaction volume** | **25 µl** |

**Table 4.** Recommended ROX concentration for PCR platforms (product not provided in this kit). If you are interested in the ROX use for quantitative PCR you may purchase the Sigma product code R4526.

| PCR platform | Recommended ROX concentration | Volume 10x ROX / 25 µl reaction |
|---|---|---|
| **Bio-Rad:** CFX96TM & CFX348TM, iQTM5 & MyiQTM, Chromo4TM, Opticon® 2 & MiniOpticon®<br><br>**Qiagen:** Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000<br><br>**Eppendorf:** Mastercycler®: ep realplex2 & ep realplex4<br><br>**Roche:** LightCycler® 480 ,LightCycler® 1,5, 2<br><br>**Illumina:** The Eco™<br><br>**Cepheid:** SmartCycler® II | No ROX | None |
| **Applied BiosSystems**: ViiA™ 7, 7500, 7700<br><br>**Stratagene:** MX3000P™, MX3005P™, MX4000P™ | 0.1x final | 0.25 µl of 10x ROX |
| **Applied BiosSystems**: 5700, 7000, 7300, 7700, 7900, 7900HT Fast, StepOne™ & StepOnePlus™ | 1x final | 2.5 µl of 10x ROX |

**Table 5.** Cycling protocol

| Step | Temperature | Time | Comments |
|---|---|---|---|
| Initial PCR activation step | 95ºC | 15 min | Activation of DNA Polymerase |
| 2-step cycling: | | | |
| Denaturation | 94ºC | 15 s | |
| Annealing | 54ºC (⁎) | 30 s | |
| Elongation | 72ºC | 45 s | |
| | 87ºC | 15 s | Fluorescent detection |
| Number of cycles | 45 | | |

| Step | Temperature | Time | Ramp Rate (ºC/s) | |
|---|---|---|---|---|
| Melting curve | 95ºC | | 20 | |
| | 65ºC | 15 s | 20 | |
| | 95ºC | | 0.1 | **Continuous** |

(⁎) Annealing temperature can be adjusted ± 2ºC, depending on the qPCR platforms

## 2.3. Analyzing the results

Interpretations of results must be done using the thermal cycler's software by trained personnel.

A positive result needs a clear amplification plot with the fluorescence curve clearly set above the threshold, but also a melting peak (Tm) of PCR product lying around 92ºC.

Before the final data interpretation, the results of the negative and positive controls must be checked in order to discard contamination and confirm proper reaction yield.

Positive controls should give positive amplification with a cycle threshold value < 26; and Tm value around 92ºC. Negative controls shouldn't give a positive amplification plot but showing Tm value around 81ºC.

Table 6 summarizes possible outcomes of the reaction. Inhibition of the PCR due to the presence of inhibitors in the samples is typically indicated by no detectable Tm value of the positive and internal control.

The melting peak of positive samples may vary according to the *Bifidobacterium* specie and the buffer used for DNA elution during purification. If the initial target DNA concentration is high, there might be only one peak (around 92ºC), and if the DNA concentration is low, there might be two peaks (81ºC of internal control, and around 92ºC to target sequence).

**Table 6.** Summary of possible outcomes

| Positive amplification plot | Melting peak (92ºC) | Melting peak (81ºC) | Result |
|---|---|---|---|
| + | + | + | Sample is positive |
| + | + | - | |
| - | - | + | Sample is negative |
| - | - | - | PCR inhibition |

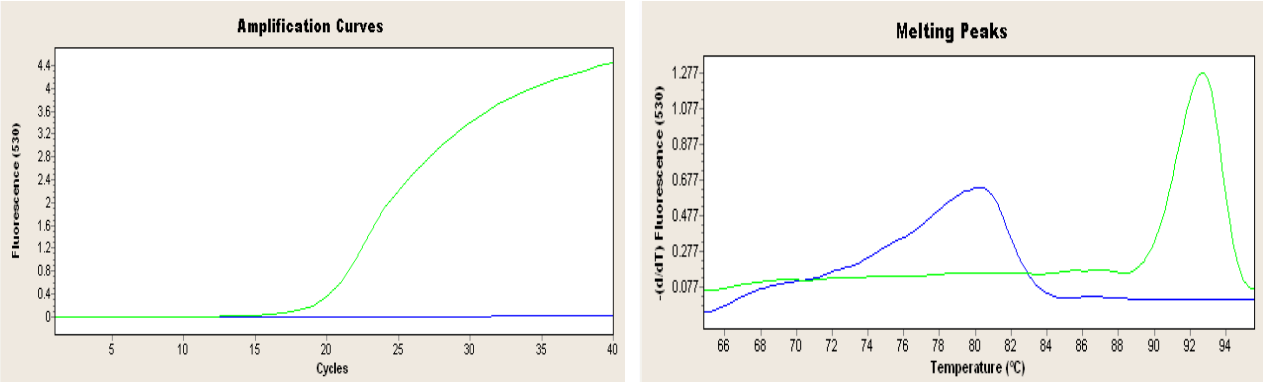


**Figure 1.** Green plots shows the positive amplification curve and 92ºC melting peak from a positive sample. Blue plots show the negative amplification curve and 81ºC melting peak from a sample without target.

In the event of PCR inhibition, dilute the DNA 1:10 with RNase-free water and repeat the test.

## 2.4 Understanding vPCR results

Although the aim of any vPCR procedure is to be near of 100% of efficiency in DNA neutralization from dead cells, the natures of samples or the cell status not always make it possible. This difficulty and the overcoming strategies have been deeply discussed in a review [1] in which our R&D was involved. If your workflow cannot obtain complete PCR signal reduction when all cells are dead the practical approach for avoiding this drawback or limit the bias, it must be done an indirect estimation of the minimum percentage of live cells [2] working with 3 sample aliquots.

| Aliquot | Workflow | Result |
|---|---|---|
| ① | vPCR | Theoretical live cells |
| ② | 80ºC, 30 min & vPCR | False positive live cells |
| ③ | PCR | Total |

**% live cells level =[(①-②) / ③ ] x100**

[1] http://www.ncbi.nlm.nih.gov/pubmed/22940102

[2] http://www.ncbi.nlm.nih.gov/pubmed/20632000

## 3. WARRANTY AND DISCLAIMER OF LIABILITY

GenIUL warrants that this product is free from defects in materials and workmanship up to the expiration date printed on the label provided the following is complied with:

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

(2) GenIUL does not warrant its product against any and all defects when: the defect appears 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 dealers or distributors selling its products beyond those herein expressly expressed, unless expressed in writing by a GenIUL officer.

(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 labour, 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.

(7) The specificity of the PCR primers used in this kit is based on current scientific available data, GenIUL, however, can't assure absence of false positives in some environmental samples.

## 4. TRADEMARKS AND LICENCES

This product has been released for evaluation and research purposes in a vPCR workflow.

The use of this product may be 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. Users should obtain the license if required.

FIREPol® is a registered trademark of Solis BioDyne. This product is sold under agreement between GenIUL and Solis BioDyne.

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.

## 5. 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 encourage you to contact us should you have any 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 Sant Nebridi 22
08222 Terrassa (Barcelona)
Spain
T.: (+34) 936193012 / F.: (+34) 932740144
geniul@geniul.com