GOODGUT
Enhancing digestive health

CE IVD

TestUrGut qPCR Kit

Intestinal Dysbiosis qPCR Kit

INSTRUCTIONS FOR USE

Reference Number REF

TestUrGut qPCR Kit: TUG-01-1024

The **TestUrGut qPCR kit** is an *in vitro* diagnostic device for use by the laboratory professional (professional user).

Intended purpose

The TestUrGut qPCR kit diagnoses gut dysbiosis by qPCR detection of specific microbial markers in stool samples.

The test allows to know the patient's intestinal health and diagnose intestinal dysbiosis. The panel of markers included in the TestUrGut qPCR kit is composed of 15 microbial markers belonging to different functional groups (proteolytic, balance, immunoprotective, muconutritive, pro-inflammatory, methanogenic, and yeast). They are represented by the following microbial groups: Eubacteria, *Escherichia coli, Faecalibacterium prausnitzii*, *Ruminococcus* spp., *Akkermansia muciniphila, Methanobrevibacter smithii, Clostridium* cluster I*, Enterococcus* sp., *Roseburia* sp., *Clostridium* cluster XIV, Gammaproteobacteria, *Lactobacillus* sp., Bacillota, Bacteroidota, and *Candida albicans*. The TestUrGut qPCR kit allows to amplify and quantify the characteristic gene fragments of the microorganisms. The results are provided quantitatively through an easy-to-interpret report. **The product is not automatic. The intended user is the laboratory professional.**

Principles of the test

The TestUrGut qPCR kit has been optimised by using multiplex qPCRs and using primers and fluorescent probes. The resulting qPCR kit is an easy-to-use tool that delivers reproducible results with high sensitivity and specificity, and a broad dynamic range. The product is based on the 5' exonuclease activity of the enzyme DNA polymerase. During DNA amplification, this enzyme cuts the probes attached to the complementary DNA sequence, separating the *quencher* from the *reporter*. This reaction generates an increase in the fluorescence signal proportional to the amount of target sequence being hydrolysed. This fluorescence can be measured on qPCR platforms.

The TestUrGut qPCR kit allows the analysis of 6 multiplex combinations per sample, necessary to obtain a diagnosis. Each kit allows a total of 24 reactions to be performed from each of the 6 multiplexes. The kit provides 6 tubes in solution (hereinafter megamix), each with the necessary reagents to analyse each of the 6 multiplexes (primers, probes, master mix, RNase-free water), ready to be used. Also provided are 6 tubes with the positive controls ready to be used, which allow to check the correct performance of each qPCR analysis.

Requirements for the TestUrGut analysis

The TestUrGut qPCR kit has been optimised to perform the quantification of the markers included in the TestUrGut microbial panel from DNA extracted from stool samples. For the analysis to be suitable, the following requirements must be met:

- Stool samples must belong to individuals of legal age (≥ 18 years old).
- Stool samples should be free of antibiotics for the month prior to bowel movements.
- Stool samples must belong to individuals who have not had a colonoscopy in the month prior to the bowel movement.
- Stool samples should come from individuals who have not undergone any surgical resection of the gastrointestinal tract.
- Not suitable for pregnant women.
- Not suitable for people diagnosed with Crohn's disease or ulcerative colitis.

- It is not recommended to perform the test during menstruation.
- Stool samples should be treated within the first 48 hours after bowel movements if they are not frozen before.

Note: *after receipt, the sample must be homogenised using a sterile spatula and then the DNA must be extracted. If DNA extraction cannot be performed upon receipt of the sample, it should be frozen at -20°C/-80°C.*

Kit contents

The TestUrGut qPCR kit contains 6 megamix tubes, 6 tubes with positive controls, and a booklet with the Quick Start Protocol. The table with the components included in the TestUrGut qPCR Kit can be found in Annex 1.

Reagents, materials, and equipment not provided

The following list includes the reagents, materials, and equipment that are required for the analysis of the TestUrGut that are not included in the TestUrGut qPCR kit:

- Sterile or single-use spatula
- DNA extraction kit (for compatibility check, see Annex 2)
- Thermal cycler (for compatibility, see Annex 3)
- Microcentrifuge Tubes
- PCR or qPCR tube strips and tube strips optical caps (8 x strip)
- Tips with filter
- Vortex Stirrer
- Centrifuge for 1.5 mL tubes
- Micropipettes (0.5 – 10 µL, 10 – 100 µL, and 100 – 1000 µL)
- Disposable Powder-Free Gloves
- Tube Strip Centrifuge

Transport and storage conditions

The TestUrGut qPCR kits are shipped in refrigeration (2-8ºC). Upon arrival, the reagents should be stored between -15ºC and -30ºC in a freezer of constant temperature and protected from light. The reagents can be frozen and thawed up to 10 times. If it is considered that more defrosts will be carried out, it is recommended to prepare several aliquots of each reagent. Once opened, the kit should be stored at -30ºC to -15ºC and can be used until the expiration date indicated on the label.

Stability in use

Storage conditions: from -30ºC to -15ºC, *see the section on transport and storage conditions.*

Shelf life after opening the reagents: see what the packaging of each of the included reagents (megamix and positive controls) indicates. After opening them, their stability is maintained until the expiration date indicated on the label, if the product is stored from -30ºC to -15ºC. Outside this temperature range, the product can remain for a maximum of 24 hours between 2ºC and 8ºC without altering its specifications.

Conditions during use: the reagents included in the kit can be kept for a maximum of 48 hours between 2-8ºC during use.

Freezing and thawing cycles with aliquots positive controls: *see transport and storage conditions.*

Safety information

- For professional use only (for professional users only).
- Do not use after the expiry date.

- Design a one-way workflow. You must start working in the Extraction Area and then move on to the Amplification and Detection Area. **Do not return the samples, equipment, and reagents to the area where the previous step was performed**.
- Good Laboratory Practices must be followed. Wear protective clothing, disposable gloves, protective goggles, and a mask. Do not eat, drink, or smoke in the work area. Once the analysis is complete, wash your hands.
- Dispose of qPCR consumables and reagents in a biowaste container.
- Regular decontamination of the equipment with which you work, especially micropipettes and work surfaces, is recommended.

Note: *There are no specific risks for the professional user, except for the usual precautions in an analysis laboratory.*

Caution: *DO NOT add bleach or acid solutions directly to sample preparation waste.*

Information on interfering substances

See section of requirements for the analysis of the TestUrGut qPCR Kit on page 1.

Quality control

According to GoodGut's Quality Management System (certified with the ISO13485), each batch of the TestUrGut qPCR kit is tested under predetermined specifications to ensure activity, efficiency, and sensitivity. The quality certificate can be found in the professional area of the GoodGut website: https://professionalarea.goodgut.eu/.

Limitations of use

The reagents in this kit are designed to work entirely with this qPCR kit. **It is not recommended to use it for other tests.** These reagents are suitable for the following instruments: see Annex 3.

So far, it has not been detected that the product contains other components that could influence the measurements.

TestUrGut qPCR kit accessories

The GoodGut-Test™ (https://goodgut-test.eu) web platform should be used to obtain the diagnosis of TestUrGut. Access to the platform is provided separately when purchasing the TestUrGut qPCR kit. The user manual is provided along with a DEMO of how the web platform works for professional laboratory users. If you have not received it, please contact support@goodgut.eu.

The recommended computer configuration for the use of the GoodGut-Test™ web platform is detailed in Table 1.

Table 1. *Recommended computer configuration for the use of the GoodGut-Test™ web platform.*

	For WINDOWS	For MAC
Scale	125%	125%
Screen Resolution	1920 x 1080	1920 x 1080
Screen Orientation	Horizontal	Horizontal

Internet access is required to use the GoodGut-Test™ web platform. It can be used with Google Chrome, Google Edge, and Mozilla Firefox browsers.

Reference measurement procedure

To ensure the correct performance of the TestUrGut qPCR kit, positive controls of known concentration are included. Positive controls should be aggregated at each run of the multiplex qPCR assay (as detailed in the protocol section of the TestUrGut qPCR kit, page 6). In addition, a non-template DNA or negative control (NTC) is required to ensure that the multiplex qPCR assay run is not contaminated.

Positive control

Positive control is used to ensure the correct performance of the qPCR run. Once the analysis parameters have been established, the Ct value obtained for the positive control must be within the range of Ct established in the 'TestUrGut Technical Specifications'. When the Ct value of the positive control is outside the accepted range, the results are not reliable. The GoodGut-Test™ web platform informs whether positive controls are accepted or rejected. If positive controls are rejected, the sample analysis should be repeated.

Control without template DNA (NTC)

NTC is used to ensure that the reaction megamix is not contaminated. Once the analysis parameters have been established, the Ct obtained in the NTC must be higher than the accepted limit value established in the 'TestUrGut Technical Specifications'. When the Ct value of the NTC is lower than the accepted limit value of Ct, the results are not reliable. The GoodGut-Test™ web platform informs whether NTC are accepted or rejected. If the NTC are rejected, the sample analysis should be repeated.

Note: *The TestUrGut technical specifications of the kit lot and the user manual of the GoodGut-Test™ web platform will be provided separately once the kit is purchased and can also be found in the professional area of the GoodGut website: https://professionalarea.goodgut.eu/.*

Reagent Information

Table 2. *Information on the reagents included in the TestUrGut qPCR kit.*

Component	Description
Megamix TUG_1	
Master mix	DNA polymerase is a modified form of a 94 kDa recombinant DNA polymerase isolated from *Thermus aquaticus*. DNA polymerase is provided in an inactive state and without enzymatic activity at room temperature. The enzyme is activated by incubating it for 1 minute at 95ºC. It contains Tris-HCl, KCl, NH_4Cl, $MgCl_2$ and additives that promote fast cycles. Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality.
RNase-free water	RNase-free distilled water for use in molecular biology.
Primers (*forward* and *reverse*)	Contains 3 sets of desalted purified primers pre-filled in the TestUrGut Multiplex 1 tube.
Probes	Contains 3 purified probes using HPLC pre-filled in the TestUrGut Multiplex 1 tube.
Megamix TUG_2	
Master mix	DNA polymerase is a modified form of a 94 kDa recombinant DNA polymerase isolated from *Thermus aquaticus*. DNA polymerase is provided in an inactive state and without enzymatic activity at room temperature. The enzyme is activated by incubating it for 1 minute at 95ºC. It contains Tris-HCl, KCl, NH_4Cl, $MgCl_2$ and additives that promote fast cycles. Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality.
RNase-free water	RNase-free distilled water for use in molecular biology.
Primers (*forward* and *reverse*)	Contains 3 sets of desalted purified primers pre-filled in the TestUrGut Multiplex 2 tube.

Component	Description
Probes	It contains 3 probes purified using HPLC pre-filled in the tube of TestUrGut Multiplex 2.
Megamix TUG_3	
Master mix	DNA polymerase is a modified form of a 94 kDa recombinant DNA polymerase isolated from *Thermus aquaticus*. DNA polymerase is provided in an inactive state and without enzymatic activity at room temperature. The enzyme is activated by incubating it for 1 minute at 95ºC. It contains Tris-HCl, KCl, NH_4Cl, $MgCl_2$ and additives that promote fast cycles. Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality.
RNase-free water	RNase-free distilled water for use in molecular biology.
Primers (*forward* and *reverse*)	Contains 3 sets of desalted purified primers pre-filled in the TestUrGut Multiplex 3 tube.
Probes	It contains 3 purified probes using HPLC pre-filled in the tube of TestUrGut Multiplex 3.
Megamix TUG_4	
Master mix	DNA polymerase is a modified form of a 94 kDa recombinant DNA polymerase isolated from *Thermus aquaticus*. DNA polymerase is provided in an inactive state and without enzymatic activity at room temperature. The enzyme is activated by incubating it for 1 minute at 95ºC. It contains Tris-HCl, KCl, NH_4Cl, $MgCl_2$ and additives that promote fast cycles. Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality.
RNase-free water	RNase-free distilled water for use in molecular biology.
Primers (*forward* and *reverse*)	Contains 2 sets of desalted purified primers pre-filled in the TestUrGut Multiplex 4 tube.
Probes	It contains 2 probes purified using HPLC pre-filled in the tube of TestUrGut Multiplex 4.
Megamix TUG_5	
Master mix	DNA polymerase is a modified form of a 94 kDa recombinant DNA polymerase isolated from *Thermus aquaticus*. DNA polymerase is provided in an inactive state and without enzymatic activity at room temperature. The enzyme is activated by incubating it for 1 minute at 95ºC. It contains Tris-HCl, KCl, NH_4Cl, $MgCl_2$ and additives that promote fast cycles. Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality.
RNase-free water	RNase-free distilled water for use in molecular biology.
Primers (*forward* and *reverse*)	Contains 2 sets of desalted purified primers pre-filled in the TestUrGut Multiplex 5 tube.
Probes	It contains 2 probes purified using HPLC pre-filled in the tube of TestUrGut Multiplex 5.
Megamix TUG_6	
Master mix	DNA polymerase is a modified form of a 94 kDa recombinant DNA polymerase isolated from *Thermus aquaticus*. DNA polymerase is provided in an inactive state and without enzymatic activity at room temperature. The enzyme is activated by incubating it for 1 minute at 95ºC. It contains Tris-HCl, KCl, NH_4Cl, $MgCl_2$ and additives that promote fast cycles. Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality.
RNase-free water	RNase-free distilled water for use in molecular biology.
Primers (*forward* and *reverse*)	Contains 2 sets of desalted purified primers pre-filled in the TestUrGut Multiplex 6 tube.
Probes	It contains 2 purified probes using HPLC pre-filled in the tube of TestUrGut Multiplex 6.

Component	Description
Positive TUG_1 control Positive TUG_2 control Positive TUG_3 control Positive TUG_4 control Positive TUG_5 control Positive TUG_6 control	Each contains a different mix of qPCR amplification products depending on the qPCR assay to be performed. They go through a thorough control process that includes verification of the measurement by capillary electrophoresis and identification of the sequence by mass spectrometry.

TestUrGut qPCR Kit protocol

To obtain the results of the TestUrGut, this protocol must be followed.

- **Sample treatment**

  Faecal samples should be treated within the first 48 hours after sample collection. Upon receipt, the sample must be homogenised with a sterile spatula and then proceed to DNA extraction. If DNA extraction cannot be performed at the time of receipt, the sample should be frozen at -80ºC if the 48 hours have not elapsed.

  The results obtained with the TestUrGut qPCR kit are only reliable when using the compatible DNA extraction kit and/or automatic extractor (for compatibility check, see Annex 2).

  The sample information must be entered on the GoodGut-Test™ web platform (https://goodgut-test.eu/) following the User Manual provided to the user once the TestUrGut qPCR kit has been purchased and in the professional area of the GoodGut website (https://professionalarea.goodgut.eu/). The sample information includes the requirements that must be met to be suitable for analysis and a sample code to correctly track its traceability.

- **qPCR protocol**

  The qPCR reactions of the 6 multiplexes have been optimised for optimal test performance and specificity. For each multiplex qPCR assay, a tube with the megamix is available.

  To obtain the diagnosis, 6 multiplex qPCRs (Multiplex TUG_1, Multiplex TUG_2, Multiplex TUG_3, Multiplex TUG_4, Multiplex TUG_5 and Multiplex TUG_6) must be performed for each sample.

  1. Determine and separate the number of tube strips and tube caps needed to perform the required reactions considering the samples and controls of each qPCR analysis (tube strips and caps are not included in the kit). A positive control and a negative no-template control (NTC) must be included in every qPCR analysis.

     ***Note**: Each qPCR Multiplex has its own positive control.*

  2. Thaw the megamix tubes and positive controls included in the qPCR kit.

  TestUrGut Multiplex TUG_1-TUG_6 qPCR Test. For the analysis of each multiplex qPCR included in the TestUrGut, steps 3 – 5 must be performed separately using the reagents reserved for each of them.

  1. Mix the megamix tube vigorously for a few seconds. Dispense 18 µL of megamix into each well (recommended by the manufacturer of the thermal cycler to be used).
  2. Add 2 µL of DNA sample into the wells containing the megamix. Add 2 µL of the specific positive control for the Multiplex analysis being performed (Positive Control TUG_1 for the Multiplex TUG_1, Positive Control TUG_2 for the TUG_2 Multiplex, Positive Control TUG_3 for the TUG_3 Multiplex, Positive Control TUG_4 for the TUG_4 Multiplex, to the wells intended for the positive controls. Positive Control TUG_5 for the Multiplex TUG_5 and Positive Control TUG_6 for the Multiplex TUG_6). Leave a well

with the megamix of the multiplex reaction being analysed, without template DNA, as a negative control (NTC) for each multiplex.

3. Close the tube strips with the corresponding caps.
4. Insert the qPCR tubes into the thermal cycler in real time.
5. Program the thermal cycler as set out in Table 3.

Note: *select the fluorescence channels (DYE) so that fluorogenic data acquisition can be performed during the combined binding/elongation phase: FAM, HEX, and ROX for the Multiplex TUG_1, Multiplex TUG_2, and Multiplex TUG_3; FAM and HEX for the TUG_4 Multiplex and TUG_5 Multiplex; and finally, FAM and ROX for the Multiplex TUG_6.*

Table 3. *Thermal cycler protocol for TestUrGut multiplex qPCR analysis.*

Step		Time (min:s)	Temperature (ºC)
Activation of qPCR		01:00	95
40 cycles	Denaturation	00:15	95
	Bonding + Elongation	00:30	60

6. Start the run.

Note: *For qPCR analysis, the following premises must be met:*

- *It is recommended that all samples and controls of the same type of qPCR assay (Multiplex TUG_1, Multiplex TUG_2, Multiplex TUG_3, Multiplex TUG_4, Multiplex TUG_5, or Multiplex TUG_6) are analysed in the same qPCR run.*
- *Do not use different kits to test the same samples. Use the same TestUrGut qPCR kit for analysis of all multiplexes with a maximum of 22 samples.*
- *For each kit, the Positive Control included in that kit should be used, do not reuse positive controls from other kits.*

- **Analysis and interpretation of the results**

1. **Perform data analysis.** The analysis of the samples is carried out using the program of the qPCR equipment used and following the manufacturer's instructions for use. The analysis of the samples is performed by setting the *Threshold* manually following the instructions for use.

   Note: *before performing the data analysis, select the preset analysis parameters defined in the 'TestUrGut Technical Specifications' (this information is provided once the TestUrGut qPCR kit is purchased and can be found in the Professional Area of the GoodGut website https://professionalarea.goodgut.eu/).*

2. To obtain the TestUrGut diagnosis, the results obtained in the analysis of each of the multiplex qPCR (including positive and negative controls) must be entered into the GoodGut-Test™ (https://goodgut-test.eu/) web platform following the User Manual.

For any technical support queries, please contact support@goodgut.eu.

In the event of an incident, defined as any fault or problem that has occurred in this *In Vitro* Medical Device during its use or subsequently, and that may have serious consequences for health, please contact the manufacturing laboratory: GoodGut S.L.U. e-mail: vigilance@goodgut.eu and/or the competent authority where the user and/or patient is established.

Description of symbols:

REF	Reference or catalogue number
VOL	Amount of liquid or reagent in a vial or vial
	Read the instructions

TestUrGut qPCR kit
Intestinal Dysbiosis qPCR kit
Basic UDI-DI: 8437023437TUGCE

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IFU-TestUrGut-001 v3.0

The information provided herein may vary due to ongoing technological updates to the product.

ANNEX 1: Components included in the TestUrGut qPCR kit.

Reagent/Material	Description	Concentration	Colour	Quantity
Megamix TUG_1	Master Mix multiplex*	4X	Amber	1 vial (500 µL)
	TUG_1_f1 Primer	100 nM		
	TUG_1_r1 Primer	100 nM		
	TUG_1_FAM probe (contains FAM fluorochrome and *BHQ1* quencher)	120 nM		
	TUG_1_f2 Primer	300 nM		
	TUG_1_r2 Primer	300 nM		
	TUG_1_HEX probe (contains HEX fluorochrome and *BHQ1* quencher)	100 nM		
	TUG_1_f3 Primer	300 nM		
	TUG_1_r3 Primer	300 nM		
	TUG_1_ROX probe (contains ROX fluorochrome and *BHQ2* quencher)	150 nM		
	RNase-free water	NA		
Megamix TUG_2	Master Mix multiplex*	4X	Amber	1 vial (500 µL)
	TUG_2_f1 Primer	250 nM		
	TUG_2_r1 Primer	250 nM		
	TUG_2_FAM probe (contains FAM fluorochrome and *BHQ1* quencher)	100 nM		
	TUG_2_f2 Primer	250 nM		
	TUG_2_r2 Primer	250 nM		
	TUG_2_HEX probe (contains the HEX fluorochrome and the *BHQ1* quencher)	250 nM		
	TUG_2_f3 Primer	150 nM		
	TUG_2_r3 Primer	150 nM		
	Probe TUG_2_ROX (contains ROX fluorochrome and *BHQ2* quencher)	150 nM		
	RNase-free water	NA		

Reagent/Material	Description	Concentration	Colour	Quantity
Megamix TUG_3	Master Mix multiplex*	4X	Amber	1 vial (500 µL)
	Primer TUG_3_f1	300 nM		
	Primer TUG_3_r1	300 nM		
	TUG_3_FAM probe (contains FAM fluorochrome and *BHQ1* quencher)	300 nM		
	TUG_3_f2 Primer	200 nM		
	TUG_3_r2 Primer	200 nM		
	Probe TUG_3_HEX (contains the fluorochrome HEX and quencher BHQ1)	200 nM		
	Primer TUG_3_f3	125 nM		
	Primer TUG_3_r3	125 nM		
	TUG_3_ROX probe (contains ROX fluorochrome and *BHQ2* quencher)	125 nM		
	RNase-free water	NA		
Megamix TUG_4	Master Mix multiplex*	4X	Amber	1 vial (500 µL)
	TUG_4_f1 Primer	250 nM		
	Primer TUG_4_r1	250 nM		
	TUG_4_FAM probe (contains FAM fluorochrome and *BHQ1* quencher)	125 nM		
	Primer TUG_4_f2	300 nM		
	TUG_4_r2 Primer	250 nM		
	TUG_4_HEX probe (contains HEX fluorochrome and *BHQ1* quencher)	250 nM		
	RNase-free water	NA		
Megamix TUG_5	Master Mix multiplex*	4X	Amber	1 vial (500 µL)
	Primer TUG_5_f1	300 nM		
	TUG_5_r1 Primer	300 nM		
	TUG_5_FAM probe (contains FAM fluorochrome and *BHQ1* quencher)	300 nM		

Reagent/Material	Description	Concentration	Colour	Quantity
	TUG_5_f2 Primer	300 nM		
	TUG_5_r2 Primer	300 nM		
	Probe TUG_5_HEX (contains the fluorochrome HEX and *BHQ1* quencher)	200 nM		
	RNase-free water	NA		
Megamix TUG_6	Master Mix multiplex*	4X	Amber	1 vial (500 µL)
	TUG_6_f1 Primer	300 nM		
	TUG_6_r1 Primer	300 nM		
	TUG_6_FAM probe (contains FAM fluorochrome and *BHQ1* quencher)	300 nM		
	TUG_6_f2 Primer	300 nM		
	TUG_6_r2 Primer	300 nM		
	Probe TUG_6_ROX (contains ROX fluorochrome and *BHQ2* quencher)	300 nM		
	RNase-free water	NA		
Positive Control TUG_1	DNA resuspended with Tris-HCl pH 8.0	108-106 copies/µL*	Transparent	1 vial (24 µL)
Positive Control TUG_2	DNA resuspended with Tris-HCl pH 8.0	108-106 copies/µL*	Transparent	1 vial (24 µL)
Positive Control TUG_3	DNA resuspended with Tris-HCl pH 8.0	108-106 copies/µL*	Transparent	1 vial (24 µL)
Positive Control TUG_4	DNA resuspended with Tris-HCl pH 8.0	108-106 copies/µL*	Transparent	1 vial (24 µL)
Positive Control TUG_5	DNA resuspended with Tris-HCl pH 8.0	108-106 copies/µL*	Transparent	1 vial (24 µL)
Positive Control TUG_6	DNA resuspended with Tris-HCl pH 8.0	108-106 copies/µL*	Transparent	1 vial (24 µL)

NA: It does not apply. *Depending on the score.

ANNEX 2: Compatibility of extraction kits and automated equipment

The DNA extraction kit and automatic extractors that can be used to obtain reliable diagnoses with TestUrGut are the following (not included in the kit):

QIAGEN DNeasy Powersoil Pro DNA Extraction Kit (manual extraction)

- Kit Reference: 47014 (50 reactions); 47016 (250 reactions), QIAGEN
- Proceed according to the manufacturer's instructions.
  ***Note**: Instead of using 250-500 mg of soil in Step 1, weigh about 40 mg of stool.*

QIAcube de QIAGEN (automatic puller)

- Use QIAGEN's DNeasy Powersoil Pro DNA Extraction Kit with QIAGEN's QIAcube Connect Automatic Extractor.
- Proceed according to the manufacturer's instructions.
  ***Note**: Instead of using 250-500 mg of soil in Step 1, weigh about 40 mg of stool.*

ANNEX 3: Real-time PCR equipment compatibility

TestUrGut multiplex reactions can be performed on all qPCR thermal cyclers equipped with a low-profile block listed below.

AriaDx (Agilent technologies)

- The analysis of the samples is carried out with the software included in the PCR equipment in real time and according to the manufacturer's instructions for use.
- Before performing the data analysis, select the preset analysis configuration for each primer + probe set (e.g., reference configuration and *threshold values*) according to the "Technical Specifications of the TestUrGut qPCR kit".
- Use tube strips and caps recommended by the thermal cycler manufacturer.

CFX96 (BioRad) / CFX Opus 96 (BioRad)

- The analysis of the samples is carried out with the software included in the PCR equipment in real time and according to the manufacturer's instructions for use.
- Specifications for analysing the results using the CFX96 software:
    - Select BR White under License plate type.
    - Apply *fluorescence drift correction*.
- Before performing the data analysis, select the preset analysis configuration for each primer + probe set (e.g., reference configuration and *threshold values*) according to the "Technical Specifications of the TestUrGut qPCR kit".
- Use tube strips and caps recommended by the thermal cycler manufacturer.