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CE IVD

RAID-CRC Screen qPCR Kit

Colorectal Cancer Screening qPCR Kit

INSTRUCTIONS FOR USE

Reference number: REF

RAID-CRC Screen qPCR Kit: CRC-01-1250-01

The **RAID-CRC Screen qPCR Kit** The RAID-CRC Screen qPCR Kit is an *in vitro* diagnostic device for professional laboratory use (professional user).

Intended use

The RAID-CRC Screen qPCR Kit is intended for screening for advanced colorectal neoplasia, including precancerous lesions and colorectal cancer, in asymptomatic individuals aged 50-69 years, both inclusive, who have obtained a positive faecal immunochemical test (FIT) result, by detecting bacterial markers in DNA extracted from patient stool samples.

The in vitro diagnostic test RAID-CRC Screen qPCR kit is based on qPCR analysis of a panel of faecal bacteria indicative of both favourable and unfavourable gut health conditions. The panel is used in asymptomatic individuals aged 50-69 years, inclusive, to rule out the presence of advanced neoplasia, including precancerous lesions and colorectal cancer. The RAID-CRC Screen qPCR kit is based on patented technology that detects a combination of 6 bacterial markers tested in faecal samples: *B46, B48, Faecalibacterium prausnitzii, Gemella morbillorum, Bacteroides fragilis* and Eubacteria. The qPCR kit allows amplification and quantification of the characteristic gene fragments of the above-mentioned microorganisms. Results are provided qualitatively and quantitatively. **This product is not automated. The intended user is a laboratory professional.**

Test principles

The RAID-CRC Screen qPCR kit has been optimised for quantitative multiplex PCR (qPCR) analysis using fluorescent primers and probes. It is an easy-to-use tool that provides reproducible results with high sensitivity, specificity and a wide dynamic range. The product is based on the 5' exonuclease activity of the enzyme DNA polymerase. During DNA amplification, this enzyme cleaves the probes bound 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 real-time PCR platforms.

The RAID-CRC Screen qPCR kit requires three qPCR analyses per sample to obtain a diagnosis. A total of 250x3 reactions can be performed with each kit. The master mix is provided ready to use, in a 4X formulation that includes all components to perform qPCR. Primers and probes are provided lyophilised in separate vials. Three positive controls are also provided, in separate tubes, to check the correct performance of each qPCR assay.

Requirements for RAID-CRC Screen use

The RAID-CRC Screen qPCR kit has been optimised for the analysis of DNA extracted from stool samples that meet the following requirements:

- Stool samples must be from asymptomatic individuals aged 50-69 years inclusive (intermediate risk population for colorectal cancer).
- Stool samples must be free of antibiotics during the month prior to stool collection.
- Stool samples should come from individuals who have not had a colonoscopy in the previous month.
- Stool samples must come from individuals who have not undergone any surgical resection of any part of the digestive tract.

- Stool samples from pregnant women are not accepted.
- The test should only be applied when the patient has a positive FIT result (cut-off point of 100 ng haemoglobin per mL using the Eiken Chemical collection tube).
  Note: *In case of using another FIT cut-off point, please contact the manufacturer (support@goodgut.eu).*
- The determination of FIT should be performed with any specific kit for this function from the manufacturer Eiken Chemical and the FIT units should be expressed in ng haemoglobin per mL.
- Stool samples should be processed within 48 hours of collection. During this period, a stool sample should be collected with the Eiken Chemical FIT collector. If it is not possible to collect the sample with the FIT collector within 48 hours, the sample should be frozen until collection can proceed.
- Once the sample has been collected with the FIT collector (Eiken Chemical), it should be stored between 2°C and 8°C until the time of determination.
- After FIT determination, DNA extraction should proceed.

Note: *The FIT collection tube (Eiken Chemical) must be stored at 2°C to 8°C until extraction. DNA extraction must be performed within 18 days after sample collection with this collector. If it is not possible to perform the extraction within the first 18 days, the FIT collector tube should be frozen at -20°C until the day of analysis, after having been at least 48 hours at 2°C to 8°C.*

- For DNA extraction, as the stool sample is diluted in the FIT collector solution, a preliminary preparation has to be performed by following the steps below:
  1. Homogenise the FIT collector by several manual inversions.
  2. Transfer the contents of the FIT collector into a 1.5 mL tube (expect 1 mL to 1.5 mL).
  3. Centrifuge the 1.5 mL tubes for 10 minutes at 4,000 xG.
  4. Discard the supernatant (expect to keep 100 µL to 200 µL of the initial volume).
  5. Homogenise the pellet by pipetting.
  6. Transfer the pellet into the pellet tubes provided in the DNeasy Powersoil Pro-Kit and proceed according to the manufacturer's instructions.
     Note: *instead of using 250 mg of soil in step 1, introduce the resuspended volume of FIT collector fluid.*
- Once the DNA extraction is complete, you can proceed with qPCR, or you can store the DNA at -20°C until analysis.

These requirements are necessary for test results to fall within the established reference ranges for colorectal cancer diagnosis using the RAID-CRC Screen qPCR kit.

Contenido del kit

Tabla 1. *Componentes incluidos en el RAID-CRC Screen qPCR Kit e información de reactivos.*

RAID-CRC Screen Kit (250 reactions x3 | 10 µL/reaction)	
Multiplex Master Mix 4X **(Contains: Taq DNA Polymerase, Antibodies, *Guard*, Buffer, mix of dNTP [dATP, dCTP, dGTP, dTTP])**	3 x 625 µL
Primer SCR_A_f1	80 µL*
Primer SCR_A_r1	80 µL*
Probe SCR_A_FAM (contains fluorochrome FAM and the *quencher* BHQ1)	120 µL*
Primer SCR_A_f2	180 µL*
Primer SCR_A_r2	180 µL*

RAID-CRC Screen Kit (250 reactions x3 | 10 µL/reaction)	
Probe SCR_A_HEX (contains fluorochrome HEX and the *quencher* BHQ1)	80 µL*
Primer SCR_A_f3	180 µL*
Primer SCR_A_r3	180 µL*
Probe SCR_A_ROX (contains fluorochrome ROX and the *quencher* BHQ2)	100 µL*
Primer SCR_B_f1	180 µL*
Primer SCR_B_r1	180 µL*
Probe SCR_B_HEX (contains fluorochrome o HEX and the *quencher* BHQ1)	80 µL*
Primer SCR_B_f2	160 µL*
Primer SCR_B_r2	160 µL*
Probe SCR_B_CY5 (contains fluorochrome CY5 and the *quencher* BHQ2)	120 µL*
Primer SCR_C_f1	180 µL*
Primer SCR_C_r1	180 µL*
Probe SCR_C_FAM (contains fluorochrome FAM and the *quencher* BHQ1)	160 µL*
Positive Control A (contains a mixture of the qPCR amplification products of SCR_A)	185 µL
Positive Control B (contains a mixture of the qPCR amplification products of SCR_B)	185 µL
Positive Control C (contains a mixture of the qPCR amplification products of SCR_C)	185 µL
RNase-free water	3 x 1.9 mL

* Volume indicated to resuspend the lyophilised oligonucleotide with Tris-HCl pH 8.0 to obtain a concentration of 2.5 µM for multiplex SCR_A marker 1 tubes and 5.0 µM for the rest of the markers.

Reagents, materials, and Equipment not supplied

The following list includes reagents, materials, and equipment that are required for the analysis of RAID-CRC Screen but are not included in the RAID-CRC Screen qPCR Kit.

- Eiken Chemical FIT collection tube
- DNA extraction kit (to check compatibility see Annex 1)
- Thermocycler and/or Real-Time PCR instrument (to check compatibility see Annex 2)
- Tris-HCl pH 8.0 Buffer (for primers resuspension)
- Microcentrifuge tubes
- Strips tubes for PCR or qPCR and optical tube strips caps (8 x strip)
- Filter tips
- Biosafety cabins
- Vortex
- Centrifuge for 1.5 mL tubes
- Micropipettes (0.5 – 10 µL, 10 – 100 µL, and 100 – 1000 µL)
- Powder-free disposal gloves

Transport and storage conditions

RAID-CRC Screen qPCR kits are shipped under cold conditions (2-8ºC). Upon arrival, the master mix and positive controls must be stored at -30°C to -15°C in a constant temperature freezer and protected from light. The reagents included in the RAID-CRC qPCR Kit can be frozen and thawed up to 10 times, if further thawing is considered, multiple aliquots of each reagent are recommended. Primers and lyophilised probes can be stored at room temperature until resuspended in Tris-HCl buffer pH 8.0. Once resuspended, they should be stored at -30°C to -15°C in a constant temperature freezer and protected from light.

Stability use

Storage conditions: Between -30°C and -15°C, see section on transport and storage conditions.

Shelf life after opening the primary container/package: the indicated on the package should be considered. After opening the container of master mix, positive controls and oligonucleotides, the stability of the product is maintained until the expiration date indicated on the packaging if the product is stored between -30ºC and -15ºC. Outside this temperature range, the product can remain for a maximum of 24 hours at 4ºC without altering its specifications.

Freezing and thawing cycles of positive control aliquots: *see transport and storage conditions.*

Safety information

- For professional use only (for professional users only).
- Do not use after expiration date.
- Design a unidirectional workflow. Start working in the Extraction Area and then move to the Amplification and Detection Area. **Do not return samples, equipment and reagents to the area where the previous step was performed.**
- Perform the mix preparation in a biosafety cabinet free of DNA. **Avoid loading the sample and positive controls on the plate in the same cabinet.**
- Good Laboratory Practices should be followed. Wear protective clothing, disposable gloves, goggles and face mask. Do not eat, drink or smoke in the work area. Once the analysis is finished, wash hands.
- Dispose of consumables and qPCR reagents in the biological waste container.
- Regular decontamination of the equipment you are working with, especially micropipettes and work surfaces, is recommended.

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

> ***CAUTION****: DO NOT add bleach or acidic solutions directly to the sample preparation waste.*

Information on interfering substances:

See RAID-CRC analysis requirements section on page 1.

Quality Control

In accordance with GoodGut's Quality Management System (ISO13485 certified), each lot of the RAID-CRC Screen qPCR Kit is tested under predetermined specifications to ensure activity, efficiency and sensitivity. The certificate of analysis can be found in the professional area of the GoodGut website: https://professionalarea.goodgut.eu/.

Reagents information

Table 2. Information of the reagents included in the RAID-CRC Screen qPCR Kit.

Component	Description
Multiplex Master Mix 4X	Contains DNA Polymerase, buffers qPCR Multiplex y Mix dNTP.
ADN Polymerase	DNA polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. DNA Polymerase is provided in an inactive state and has no enzymatic activity at room temperature. The enzyme is activated by a 1-minute, 95ºC incubation step.
qPCR Multiplex Buffer	Contains Tris-HCl, KCl, NH_4Cl, $MGCl_2$ and additives enabling fast cycling.
Mix dNTP	Contains dATP, dCTP, dGTP y dTTP of ultra-pure quality.
Primers (*forward y reverse*)	Contains 6 primers sets purified using HPLC.
Probes	Contains 6 probes purified using HPLC.
Positive Controls SCR	Contains a pool of 6 qPCR amplification products, which go through a quality control process including size verification by capillary electrophoresis and sequence identification by mass spectrometry.

Limitations of use:

The reagents in this kit are designed to work fully with this qPCR kit. **It is not recommended to use them for other tests.** These reagents are suitable for the following instruments (see Appendix 2).

To date, no other components have been found in the product that could influence measurements.

RAID-CRC Screen qPCR Kit accessories

GoodGut-Test™ web platform (https://goodgut-test.eu) must be used to obtain the RAID-CRC Screen diagnostic. The access to the platform is provided separately when the RAID-CRC Screen qPCR product is acquired. The user manual is provided together with a DEMO of how the web platform works to professional laboratory users. If you haven't received it, please contact support@goodgut.eu.
The recommended computer configuration for the use of the GoodGut-Test™ web platform is detailed in Table 3.

Table 3. Recommended computer configuration for the use of 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 proper performance of the RAID-CRC Screen qPCR kit, positive controls of known concentration are included. Positive controls must be added to each multiplex qPCR assay run (as detailed in the RAID-CRC Screen qPCR kit protocol, page 8). In addition, a no-template DNA control or negative control (NTC) is required to ensure that the multiplex qPCR assay run is not contaminated.

Positive control

The positive control is used to ensure proper performance of the qPCR run. Once the analysis parameters have been established, the *Ct* value obtained for the positive control must fall within the Ct range established in the RAID-CRC Screen qPCR Kit Technical Specifications. When the *Ct* value of the positive control falls outside the accepted range, the results are unreliable. The GoodGut-Test™ web platform reports whether the positive controls are accepted or rejected. If the positive controls are rejected, the sample analysis must be repeated.

The tolerance range for positive controls is available to all customers in the lot-specific RAID-CRC Screen qPCR Kit technical specifications provided upon purchase. The technical specifications can also be found in the professional area of the GoodGut website: https://professionalarea.goodgut.eu/.

No Template Control (NTC)

The no template control, or negative control (NTC), is used to ensure that the reaction mix is not contaminated. A specific cutoff value is defined for each lot of the RAID-CRC Screen qPCR Kit.
After analysis and interpretation of results, the *Ct* value obtained on the NTC must be higher than the cutoff value established for each lot. When the NTC *Ct* value is lower than the established cutoff value, the results are unreliable. The GoodGut-Test™ web platform reports whether the NTCs are accepted or rejected. If the NTCs are rejected, the sample analysis must be repeated.

The NTC *cutoff* value is available to all customers in the lot-specific RAID-CRC Screen qPCR Kit technical specifications provided at the time of purchase. Technical specifications can also be found in the professional area of the GoodGut website: https://professionalarea.goodgut.eu/.

RAID-CRC Screen qPCR Kit Protocol

This protocol must be followed to obtain RAID-CRC Screen results.

- **Sample treatment**
  Faecal samples must be processed within the first 48 hours after sample collection or frozen until processing is possible. Upon arrival, the sample must be collected using the Eiken Chemical FIT collector and stored between 2°C and 8°C. The FIT value is analysed at least 48 hours later. Once the FIT value is known, DNA can be extracted. Up to 18 days can elapse between sample collection with the FIT collector tube and extraction, provided the sample is stored between 2°C and 8°C. The FIT collector tube can be frozen at -20°C after a minimum of 48 hours between 2°C and 8°C, until DNA extraction is performed, if it is necessary to exceed 18 days.

  The extracted DNA must be stored at -20°C until the day of qPCR analysis. Once the DNA sample has been used, it can be frozen again at -20°C. The DNA sample can be thawed and frozen a maximum of 5 times.

  Results obtained with the RAID-CRC Screen qPCR Kit are only reliable when using compatible DNA extraction kits and/or automated extractors (see Annex 1 for compatibility).

  Sample information must be entered into the GoodGut-Test™ web platform (https://goodgut-test.eu/) following the User Manual provided to the user upon purchase of the RAID-CRC Screen qPCR Kit and in the professional area of the GoodGut website (https://professionalarea.goodgut.eu/). Sample information includes the requirements that must be met to be eligible for analysis and a sample code for proper traceability.

- **qPCR protocol**
  The number of primers/probes and sample, as well as the parameters (temperature (hybridization), number of cycles and time of each step) have been optimized for optimal performance and specificity of the assay.
  Before starting, the primers and probes should be resuspended with the volume of Tris-HCl pH 8.0 indicated in the protocol (Kit Contents section).

Note: *for optimal resuspension of the primers and probes, after adding the Tris-HCl buffer, incubate the tubes at room temperature for 1 hour or overnight at 4°C without changing containers. Once resuspended, they should be stored at -30°C to -15°C in a freezer at a constant temperature and protected from light.*

To obtain the diagnosis, two multiplex and one singleplex qPCR analysis should be performed for each sample: Screen A (SCR_A), Screen B (SCR_B) and Screen C (SCR_C).

1. Determine and separate the number of tube strips and plugs/tubes needed to perform the required reactions considering the samples and controls for each qPCR assay (material not included in the RAID-CRC Screen qPCR kit). A positive control and a negative control without template DNA (NTC) should be included in each qPCR assay. Note: Each qPCR has its own positive control.
2. Thaw the Multiplex Master Mix 4X, primers, probes and positive controls.

Screen A multiplex qPCR analysis:

3. Add the following components into a microcentrifuge tube (Table 4). It is recommended to prepare a Mix volume of n x 1.1 (where n is the number of reactions), to minimize the effect of pipetting error. Minimize exposure of fluorescently labelled probes to light. **Note:** The number of reactions to be performed simultaneously should be equal to or less than the number of reactions that can be performed in the thermal cycler.

Table 4. *Reaction mix for multiplex qPCR analysis RAID-CRC Screen A (per reaction).*

Component	Final concentration	Volume/reaction
Multiplex Master Mix 4X	1X	2.50 µL
Primer SCR_A_f1	50 nM	0.20 µL
Primer SCR_A_r1	50 nM	0.20 µL
Probe SCR_A_FAM	60 nM	0.24 µL
Primer SCR_A_f2	300 nM	0.60 µL
Primer SCR_A_r2	300 nM	0.60 µL
Probe SCR_A_HEX	100 nM	0.20 µL
Primer SCR_A_f3	300 nM	0.60 µL
Primer SCR_A_r3	300 nM	0.60 µL
Probe SCR_A_ROX	150 nM	0.30 µL
RNase-free water	-	1.96L

4. Mix the reaction vigorously and centrifuge briefly. Dispense 8 µL into the qPCR tubes recommended by the manufacturer of the thermal cycler to be used.
5. Add 2 µL of DNA samples into the qPCR tubes containing the reaction mix. In addition, also add 2 µL of the specific positive control for the qPCR multiplex Screen A analysis to the tube reserved for this control and leave one tube with the reaction mix alone as the NTC negative control. Close the tubes with the appropriate caps.

Screen B multiplex qPCR analysis:

6. Add the following components into a microcentrifuge tube (Table 5). It is recommended to prepare a Mix volume of n x 1.1 (where n is the number of reactions), to minimize the effect of pipetting error. Minimize exposure of fluorescently labelled probes to light.
   Note: *The number of reactions to be performed simultaneously should be equal to or less than the number of reactions that can be performed in the thermal cycler.*

Table 5. Reaction mix for multiplex qPCR RAID-CRC Screen B analysis (per reaction).

Component	Final concentration	Volume/reaction
Multiplex Master Mix 4X	1X	2.50 µL
Primer SCR_B_f1	300 nM	0.60 µL
Primer SCR_B_r1	300 nM	0.60 µL
Probe SCR_B_HEX	100 nM	0.20 µL
Primer SCR_B_f2	250 nM	0.50 µL
Primer SCR_B_r2	250 nM	0.50 µL
Probe SCR_B_CY5	200 nM	0.40 µL
RNase-free water	-	2.70 µL
Total reaction volume		**8 µL**

7. Mix the reaction vigorously and centrifuge briefly. Dispense 8 µL into the qPCR tubes recommended by the manufacturer of the thermal cycler to be used.
8. Add 2 µL of DNA samples into the qPCR tubes containing the reaction mix. In addition, also add 2 µL of the specific positive control for the qPCR multiplex Screen B analysis to the tube reserved for this control and leave one tube with the reaction mix alone as NTC negative control. Close the tubes with the appropriate caps.

Screen C multiplex qPCR analysis:

9. Add the following components into a microcentrifuge tube (Table 6). It is recommended to prepare a Mix volume of n x 1.1 (where n is the number of reactions), to minimise pipetting error. Minimise exposure of fluorescently labelled probes to light.
   Note: *The number of reactions to be performed simultaneously should be equal to or less than the number of reactions that can be performed in the thermal cycler.*

Table 6: Reaction mix for RAID-CRC Screen C multiplex qPCR analysis (per reaction).

Component	Final concentration	Volume/reaction
Multiplex Master Mix 4X	1X	2.50 µL
Primer SCR_C_f1	300 nM	0.60 µL
Primer SCR_C_r1	300 nM	0.60 µL
Probe SCR_C_FAM	250 nM	0.50 µL

Component	Final concentration	Volume/reaction
RNase-free water	-	3.80 µL
Total reaction volume		**8 µL**

10. Mix the reaction vigorously and centrifuge briefly. Dispense 8 µL into the qPCR tubes recommended by the manufacturer of the thermal cycler to be used.
11. Add 2 µL of DNA samples into the qPCR tubes containing the reaction mix. In addition, also add 2 µL of the specific positive control for the qPCR multiplex Screen C analysis to the tube reserved for this control and leave one tube with the reaction mix alone as NTC negative control. Close the tubes with the appropriate caps.
12. Program the thermal cycler according to the programme in Table 7, all three qPCR assays have the same programme.
    Note: *Select specific channels (targets) for fluorogenic data acquisition to be performed during the combined binding/elongation step: FAM, HEX and ROX for Screen A; HEX and CY5 for Screen B; and FAM for Screen C.*

Table 7. Thermal cycler protocol for multiplex/singleplex qPCR RAID-CRC Screen analyses.

Step		Time (min:s)	Temperature (ºC)
qPCR activation step		01:00	95
40 cycles	Denaturation	00:15	95
	Annealing + Extension	00:30	60

13. Insert the qPCR tubes into the real-time thermal cycler and start the analysis.
    Note: *all samples and controls of the same type of qPCR analysis (Screen A or Screen B or Screen C) must be analysed on the same qPCR run. In case more than one thermal cycler is used for the analysis of a sample (e.g. Screen A analysed on thermal cycler 1 and Screen B on thermal cycler 2) make sure to use the same model.*

- **Analysis and interpretation of the results**

1. Perform the data analysis. The analysis of the samples is carried out using the software of the qPCR kit used and following the manufacturer's instructions for use.
   Note: *before performing the data analysis, select the pre-set analysis parameters for each primer + probe set (baseline and threshold values) according to the 'RAID-CRC Screen Technical Specifications' (this information is provided once the RAID-CRC Screen qPCR kit is purchased and can be found in the Professional Area of the GoodGut website https://professionalarea.goodgut.eu/).*

2. To obtain the RAID-CRC Screen diagnostic, the results obtained in the multiplex qPCR analysis (including positive and negative controls) must be entered into the GoodGut-Test™ web platform (https://goodgut-test.eu/) following the user manual. The results should be uploaded to the Platform in different Excel files specific to each multiplex which should contain the sample identifier, the dye and the raw *Ct* (Cq) value. The Excel templates can be downloaded from the platform by following the user manual.

For any technical support questions or feedback, please contact support@goodgut.eu.

In the event of an incident, defined as any malfunction or problem that may occur with this *In Vitro* Medical Device (IVD), during or after use, and which may have serious health consequences, please contact the manufacturing laboratory (GoodGut S.L.U.) (e.g., vigilance@goodgut.eu) and/or the competent authority where the user and/or patient is located.

Description of symbols:

REF	Reference or catalog number
VOL	Quantity of liquid or reagent contained in a vial or bottle
	Read the instructions

RAID-CRC Screen qPCR Kit
Colorectal Cancer Screening qPCR Kit
Basic UDI-DI: 8437023437RAIDCRCKC

GOODGUT, SLU
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The information reported in this document may vary due to continuous technological updates.

ANNEX 1: COMPATIBILITY OF THE DNA EXTRACTION KIT AND AUTOMATED EQUIPMENT

The DNA extraction kit and the automated extractors that can be used to obtain reliable diagnostics in RAID-CRC Screen are the following:

Qiagen DNeasy Powersoil Pro DNA Extraction Kit (Manual Extraction)

- Kit Reference: 47014, QIAGEN
- Before proceeding with the DNA extraction, the following instructions must be followed:
    1. Homogenise FIT collector by inverting it manually several times.
    2. Transfer FIT collector content into a 1.5 mL tube (expect between 1 mL to 1.5 mL).
    3. Centrifuge the 1.5 mL tubes for 10 minutes at 4,000 xG.
    4. Discard the supernatant (expect to keep between 100 µL to 200 µL of the initial volume).
    5. Homogenise the pellet by pipetting.
    6. Transfer the pellet into the beads tube provided in the DNeasy Powersoil Pro Kit and proceed following the manufacturer's instructions.

       ***Note**: instead of using 250 mg of soil in Step 1, introduce the volume resuspended from the FIT collector fluid.*

Qiagen QIAcube (automatic extractor)

- Use the DNeasy Powersoil Pro DNA extraction kit from Qiagen with the automated extractor QIAcube Connect from QIAGEN.
- Before proceeding with the DNA extraction, the following instructions must be followed:
    1. Homogenise FIT collector by inverting it manually several times.
    2. Transfer FIT collector content into a 1.5 mL tube (expect between 1 mL to 1.5 mL).
    3. Centrifuge the 1.5 mL tubes for 10 minutes at 4,000 xG.
    4. Discard the supernatant (expect to keep between 100 µL to 200 µL of the initial volume).
    5. Homogenise the pellet by pipetting.
    6. Transfer the pellet into the beads tube provided in the DNeasy Powersoil Pro Kit and proceed following the manufacturer's instructions.

       ***Note**: instead of using 250 mg of soil in Step 1, introduce the volume resuspended from the FIT collector fluid.*

ANNEX 2: COMPATIBILITY OF THE REAL TIME PCR EQUIPMENT

RAID-CRC Screen multiplex can be performed in the thermocyclers equipped with a low-profile block listed below.

AriaDx (Agilent Technologies)

- The analysis of the samples is performed with the software included in the real-time PCR equipment and according to the manufacturer's instructions for use.
- Before performing data analysis, select the preestablished analysis settings for each *primers + probe* set (i.e., baseline settings and threshold values) according the 'Technical specifications of RAID-CRC Screen qPCR Kit'.

CFX96 (BioRad)

- The analysis of the samples is performed with the software included in the real-time PCR equipment and according to the manufacturer's instructions for use.
- Specifications for analysing the results using CFX96 software:
    - Select BR White in plate type.
    - Apply the fluorescence drift correction.
- Before performing data analysis, select the preestablished analysis settings for each *primers + probe* set (i.e., baseline settings and threshold values) according the 'Technical specifications of RAID-CRC Screen qPCR Kit'. ***Note**: The specific 'RAID-CRC Screen Technical Specifications' for your specific lot are provided separately when acquiring the kit and are also found on the Professional area of GoodGut website https://professionalarea.goodgut.eu/.*