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CE IVD

RAID-CRC Symptomatic qPCR Kit

Colorectal Cancer Detection qPCR Kit

INSTRUCTIONS FOR USE

Reference number: REF

RAID-CRC SYMPTOMATIC qPCR Kit: CRC-01-2250-01

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

Intended Use

The RAID-CRC Symptomatic qPCR Kit is intended for the screening of advanced colorectal neoplasia in symptomatic patients who obtain a positive result in the faecal immunochemical test (FIT), through the detection of specific bacterial markers in DNA samples extracted from patients' faeces.

The RAID-CRC Symptomatic qPCR Kit *in vitro* diagnostic test is based on the qPCR analysis of a panel of faecal bacteria indicative of both favourable and unfavourable conditions of intestinal health. The panel is used in symptomatic individuals as a screening step to rule out colorectal cancer. The RAID-CRC Symptomatic qPCR Kit is based on proprietary technology that detects a combination of 4 bacterial markers tested in faecal samples: *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Peptostreptococcus stomatis*, and Eubacteria. The qPCR kit allows amplifying and quantifying the characteristic gene fragments of the mentioned microorganisms. Results are provided in qualitative and quantitative form. **The product is not automatic. The intended user is a laboratory professional.**

Test principles

The RAID-CRC Symptomatic qPCR Kit is optimised for quantitative PCR (qPCR) assays in multiplex using specific primers and fluorescent-labelled probes. It is an easy-to-use tool that offers reproducible results with high sensitivity, specificity, and broad dynamic range. This product is based on 5' exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probes bound to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of the hydrolysed target sequence. This fluorescence can be measured on real-time PCR platforms.

The RAID-CRC Symptomatic qPCR Kit requires one qPCR assays for each sample to obtain a diagnostic. A total of 250 reactions can be performed with each kit. The master mix is provided as a ready-to-use, stabilised 4X formulation that includes all components for qPCR. The primers and probes are provided lyophilised in separate tubes. A positive control is also provided in a separate tube to check the correct performance of each qPCR run

Requirements for RAID-CRC Symptomatic use

This RAID-CRC Symptomatic qPCR Kit has been optimised for the analysis of DNA extracted from faecal samples that fulfil the following conditions:

- Faecal samples must come from individuals with symptoms compatible with colorectal cancer (CRC) such as the presence of rectal bleeding, changes in bowel habits, anaemia, unexplained weight loss, diarrhoea, iron deficiency anaemia, and/or abdominal mass.
- Faecal samples must come from subjects over 18 years old.
- Faecal samples must be free of antibiotics the month prior to deposition.
- Faecal sample must come from a person who has not undergone a colonoscopy in the previous month.
- Faecal sample must come from a person who has not had surgical resections of any part of the digestive tract.

- Faecal samples from pregnant women are not accepted.
- This test must be only applied when a positive result of FIT (cut-off of 150 ng of haemoglobin per mL using the Eiken Chemical tube collector) is obtained. **Note**: In case of using another FIT cut-off value, please contact the manufacturer (support@goodgut.eu)
- FIT determination must be performed with any equipment with this specific function from Eiken Chemical. FIT units must be expressed in ng of haemoglobin per mL.
- Faecal samples must be treated within the first 48 hours after sample collection. Within this period stool sample must be collected with the Eiken Chemical FIT collector.
- Once the sample has been collected with the FIT collection tube (Eiken Chemical) should be stored at 2ºC to 8ºC until FIT analysis.
  Note: *The FIT (Eiken Chemical) collection tube must be stored between 2ºC and 8ºC until extraction. DNA extraction must be performed within the first 18 days after sample collection using this collector. If extraction cannot be performed within the first 18 days, the FIT collection tube must be frozen at -20ºC until the day of analysis, after having been stored at 2ºC to 8ºC for at least 48 hours.*
- For the DNA extraction, since the faecal sample is diluted in the FIT collector solution a preliminary sample preparation needs to be done following the next steps:
  - Homogenise FIT collector by inverting it manually several times.
  - Transfer FIT collector content into a 1.5 mL tube (expect between 1 mL to 1.5 mL).
  - Centrifuge the 1.5 mL tubes for 10 minutes at 4,000 xG.
  - Discard the supernatant (expect to keep between 100 µL to 200 µL of the initial volume).
  - Homogenise the pellet by pipetting.
  - 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.
- Once DNA extraction is complete, qPCR can be performed, or the DNA can be stored at -20ºC until analysis.

These requirements are necessary for the analysis results to be within the reference intervals established for the detection of colorectal cancer using the RAID-CRC Symptomatic qPCR kit.

Kit contents

Table 1. Components included in the RAID-CRC Symptomatic qPCR Kit.

RAID-CRC Symptomatic qPCR Kit (250 reactions | 10 µL/reaction)	
Multiplex Master Mix 4X **(Contains: Taq DNA Polimerasa, Anticuerpos, *Guard*, Buffer, dNTP mix [dATP, dCTP, dGTP, dTTP])**	1 x 625 µL
Primer SYM_f1	80 µL*
Primer SYM_r1	80 µL*
Probe SYM_FAM (contains the fluorochrome FAM and the *quencher* BHQ1)	120 µL*
Primer SYM_f2	100 µL*
Primer SYM_r2	100 µL*
Probe SYM_HEX (contains the fluorochrome HEX and the *quencher* BHQ1)	120 µL*
Primer SYM_f3	100 µL*

RAID-CRC Symptomatic qPCR Kit (250 reactions | 10 µL/reaction)	
Primer SYM_r3	100 µL*
Probe SYM_CY5 (contains CY5 and the *quencher* BHQ2)	120 µL*
Primer SYM_f4	100 µL*
Primer SYM_r4	100 µL*
Probe SYM_ROX (contains the fluorochrome ROX and the *quencher* BHQ2)	120 µL*
Positive control SYM (contains a mixture of the qPCR amplification products of SYM)	185 µL
RNase-free water	1 x 1.9 mL

* Volume indicated to resuspend the lyophilized oligonucleotide with Tris-HCl pH 8.0 and obtain a concentration of 2.5 µM for marker 1 tubes and 5.0 µM for markers 2, 3 and 4.

Reagents, materials, and Equipment not supplied

The following list includes reagents, materials, and equipment that are required for the analysis of RAID-CRC Symptomatic but are not included in the RAID-CRC Symptomatic 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 Symptomatic qPCR Kits are shipped in cool conditions (2 - 8ºC). Upon receipt the master mix and the positive control should be stored at -15ºC to -30ºC in a constant-temperature freezer and protected from light. It is recommended to make several aliquots of the positive control to avoid undergoing more than 10 freezing/thawing cycles. Lyophilised primers and probes can be stored at room temperature until being resuspended in Tris-HCl pH 8.0 buffer. Once resuspended they should be stored at -30ºC to -15ºC 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/packaging: Observe the information on the packaging. After opening the master mix, positive controls, and oligonucleotides, the product remains stable until the expiration date indicated on the packaging if stored between -30°C and -15°C. Outside this temperature range, the product can remain at 4°C for a maximum of 24 hours without altering its specifications.

Freeze-thaw cycles of aliquots of positive controls: *see transport and storage conditions.*

Safety information

- For professional *in vitro* use only (professional users only).
- Do not use after the expiration date.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. **Do not return samples, equipment, and reagents to the area in which the previous step was performed.**
- Prepare the mix in a DNA-free biosafety cabinet. **Avoid loading the sample and positive controls onto the plate within the same cabinet.**
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, protective goggles, and a mask. Do not eat, drink, or smoke in the working area. Once you finish the test wash your hands.
- Dispose of qPCR consumables and reagents in the biological waste container.
- Regular decontamination of work equipment, 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 ISO13485-certified Quality Management System, each lot of RAID-CRC Symptomatic qPCR Kit is tested against predetermined specifications to ensure activity, efficiency, and sensitivity. The quality certificate can be found on the Professional area of GoodGut website: https://professionalarea.goodgut.eu/.

Reagents information

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

Componente	Descripción
Multiplex Master Mix 4X	Contains DNA Polymerase, buffers qPCR Multiplex and Mix dNTP.
DNA 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.
Multiplex qPCR 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 4 primers sets purified using HPLC.
Probes	Contains 4 probes purified using HPLC.
Positive control SYM	Contains a pool of 4 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 Symptomatic qPCR Kit accessories

GoodGut-Test™ web platform (https://goodgut-test.eu) must be used to obtain the RAID-CRC Symptomatic diagnostic. The access to the platform is provided separately when the RAID-CRC Symptomatic 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 Symptomatic 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 Symptomatic 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 Symptomatic 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 Symptomatic 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 Symptomatic 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 Symptomatic 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 Symptomatic qPCR Kit Protocol

This protocol must be followed to obtain RAID-CRC Symptomatic 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 Symptomatic 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 Symptomatic 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 quantity of primers/probes and sample, as well as the parameters (annealing temperature, number of cycles, and time of each step) have been optimized to achieve optimal assay performance and specificity.

  Before starting, resuspend the primers and probes with the volume of Tris-HCl pH 8.0 indicated in the protocol (Kit Contents section).

  Note: *For optimal resuspension of 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 between -30°C and -15°C in a constant-temperature freezer, protected from light.*

  1. Determine and separate the number of tube strips and caps/tubes required to perform the required reactions, considering the samples and controls for each qPCR analysis (material not included in the RAID-CRC Symptomatic qPCR kit). A positive control and a negative control without template DNA (NTC) must be included in each qPCR analysis.

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

  2. Thaw the 4X Multiplex Master Mix, primers, probes, and positive controls.

3. Add the following components to 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 errors. 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 run in the thermal cycler.*

Table 4. *Reaction mix for performing the RAID-CRC Symptomatic multiplex qPCR analysis (per reaction).*

Component	Final Concentration	Volume/reaction
Multiplex Master Mix 4X	1X	2.50 µL
Primer SYM_f1	50 nM	0.20 µL
Primer SYM_r1	50 nM	0.20 µL
Probe SYM_FAM	60 nM	0.24 µL
Primer SYM_f2	150 nM	0.30 µL
Primer SYM_r2	150 nM	0.30 µL
Probe SYM_HEX	200 nM	0.40 µL
Primer SYM_f3	150 nM	0.30 µL
Primer SYM_r3	150 nM	0.30 µL
Probe SYM_CY5	200 nM	0.40 µL
Primer SYM_f4	150 nM	0.30 µL
Primer SYM_r4	150 nM	0.30 µL
Probe SYM_ROX	200 nM	0.40 µL
RNase-free water	-	1.86 µL

4. Mix the reaction gently and centrifuge it briefly. Dispense 8 µL into the qPCR tubes recommended by your thermocycler manufacturer.

5. Add 2 µL of the DNA samples to the individual qPCR tubes that contain the reaction mix. Add also 2 µL of the positive control to the tube reserved for this control and leave a tube only with the reaction mix as a no template control (NTC). Close the qPCR tubes with the optical caps provided.

6. Program your thermocycler according to Table 5.

Note: *Selection of the specific channels (targets) for fluorogenic data acquisition can be performed during the combined annealing/extension step: FAM, HEX, ROX, and CY5.*

Table 5. Thermal cycling protocol for RAID-CRC Symptomatic multiplex qPCR assay.

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

- **Analysis and interpretation of the results**

1. Perform data analysis. Sample analysis is performed using the qPCR software used and following the manufacturer's instructions for use. **Note:** Before performing data analysis, select the preset analysis parameters for each Primer + Probe System (baseline and threshold values) according to the "RAID-CRC Symptomatic Technical Specifications" (this information is provided upon purchase of the RAID-CRC Symptomatic qPCR Kit and can be found in the Professional Area of the GoodGut website (https://professionalarea.goodgut.eu/).

2. To obtain the RAID-CRC Symptomatic diagnosis, the results obtained from 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 must be uploaded to the Platform in an Excel file specific to the RAID-CRC Symptomatic multiplex, which must contain the sample identifier, the dye, and the raw *Ct* (Cq) value. The Excel template can be downloaded from the platform 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 Symptomatic qPCR Kit
Colorectal Cancer Detection qPCR Kit
Basic UDI-DI: 8437023437RAIDCRCKC

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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 Symptomatic 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 Symptomatic 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 Symptomatic 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 Symptomatic qPCR Kit'.

  ***Note**: The specific 'RAID-CRC Symptomatic 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/.*