RAID-CRC SYMPTOMATIC KIT

Colorectal Cancer Detection qPCR Kit

General description

The RAID-CRC Symptomatic Kit is optimized 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.

A total of 250 reactions can be performed with the RAID-CRC Symptomatic kit. The master mix is provided as a ready-to-use, stabilized 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.

Intended Use

The RAID-CRC Symptomatic 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.

Requirements for RAID-CRC Symptomatic use

This RAID-CRC Symptomatic kit has been optimized 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 be free of antibiotics the month prior to deposition.
- Faecal samples from pregnant women are not accepted.
- This test must be only applied when a positive result of FIT (cut-off of 10 µg haemoglobin per g of faeces) is obtained. **Note**: In case of using another FIT cut-off value, please contact the manufacturer (soporte@goodgut.eu)
- FIT determination can be performed with any FIT analyser if the units of the FIT result used for RAID-CRC Symptomatic analysis are expressed in µg of haemoglobin per g of faeces. **Note**: Although the

value obtained with any commercial brand of the FIT can be used, to proceed with the RAID-CRC Symptomatic analysis, it must be performed from a stool sample collected using the Eiken Chemical FIT collection tube.

- Within the first 48 hours of sample deposition, a stool sample must be collected with the Eiken Chemical FIT collector.
- The FIT collection tube (Eiken Chemical) should be stored at 2ºC to 8ºC until DNA extraction is performed. **Note**: DNA extraction must be performed within the first 18 days after sample collection with this collector.
- The results obtained using the RAID-CRC Symptomatic kit are only valid when the DNeasy Powersoil Pro DNA extraction Kit from Qiagen is used. **Note**: since the faecal sample is diluted in the FIT collector solution a preliminary sample preparation needs to be done following the next steps:
    1. Homogenize 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. Homogenize 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.

Kit Contents

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

RAID-CRC Symptomatic Kit (250 reactions | 10 µL/reaction)	
Multiplex Master Mix 4X (Contains: Taq DNA Polymerase, Antibody, 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 Kit (250 reactions | 10 µL/reaction)	
Primer SYM_r3	100 µL*
Probe SYM_CY5 (contains the fluorochrome 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	2 x 1.9 mL

* Volume indicated to resuspend the lyophilized oligonucleotide with Tris-HCL (pH 7.4 for primers and 8.1 for probes) to obtain a concentration of 2.5 µM for 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 Symptomatic but are not included in the RAID-CRC Symptomatic kit.

- Eiken Chemical FIT collection tube
- Thermocycler and/or Real-Time PCR instrument (to check compatibility see 'RAID-CRC Symptomatic Technical Specifications' which can be found on the GoodGut website (www.goodgut.eu) or on the GoodGut-Test® platform (https://goodgut-test.eu/auth/login))
- DNeasy Powersoil Pro DNA extraction Kit (Ref. 47014, QIAGEN)
- Tris-HCl pH 7.4 Buffer (for primers resuspension)
- Tris-HCl pH 8.1 Buffer (for probes resuspension)
- GoodGut-Test® Platform for RAID-CRC Symptomatic diagnosis (http://goodgut-test.eu/auth/login)
- Microcentrifuge tubes
- Strips tubes for PCR or qPCR and optical tube strips caps (8 x strip)
- Filter tips
- 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 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 3 freezing/thawing cycles. Lyophilised primers and probes can be stored at room temperature until being resuspended in Tris-HCl pH 7.4 buffer for primers and pH 8.1 for probes. Once resuspended they should be stored at -15ºC to -30ºC in a constant-temperature freezer and protected from light.

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.
- 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.
- Discard all the consumables and the qPCR reagents into the biological container.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and working surfaces.

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

Quality Control

In accordance with GoodGut's ISO13485-certified Quality Management System, each lot of RAID-CRC Symptomatic Kit is tested against predetermined specifications to ensure activity, efficiency, and sensitivity. The quality certificate can be found on the GoodGut website: www.goodgut.eu.

Reagents Information

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

Component	Description
Multiplex Master Mix 4X	
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

Component	Description
	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.
dNTP mix	Contains dATP, dCTP, dGTP, and dTTP of ultra-pure quality.
Primers (forward and reverse)	Contains 4 primers sets purified using HPLC.
Probes	Contains 4 probes purified using HPLC.
Positive control	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.

qPCR Protocol

This protocol must be followed to obtain RAID-CRC Symptomatic results. The amount of primers/probe and template, as well as the parameters (temperatures (annealing), cycle number and step times), has been optimized for an optimal yield and specificity of the multiplex assay.

Before starting, resuspend the primers and probes with the volumes of Tris-HCl indicated in the protocol (kit content section). In terms of the primers, Tris-HCl pH must be 7.4 and in terms of the probes, it must be 8.1. **Note**: For a proper resuspension of primers and probes, after the Tris-HCl buffer addition, incubate the tubes at room temperature for 1 hour or overnight at 4ºC. Once resuspended they should be stored at -15ºC to -30ºC in a constant-temperature freezer and protected from light.

1. Determine and separate the number of strips and caps for required reactions including samples and controls. A positive control and a no-template control (NTC) should be included in each qPCR run.
2. Thaw Multiplex Master Mix 4X, primers, probes, and the positive control.
3. Add the following components to a microcentrifuge tube (Table 1). We recommend preparing a n x 1.1 volumes of Mix (where n is the number of reactions), in order to reduce pipetting errors. Minimize the exposure of the fluorescent-labelled probe to light. **Note**: The number of reactions to be performed, at the same time, must be equal to or lower than the number of reactions permitted in the thermocycler.

Table 3. Reaction mix setup for RAID-CRC Symptomatic multiplex qPCR assay (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

Component	Final Concentration	Volume/reaction
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
Total reaction volume		**8 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 4. **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 4. 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

7. Place the qPCR tubes in the real-time thermocycler and start the run.

Analysis and Interpretation of the Results

1. **Perform data analysis**.

The analysis of the samples is done using the software of the used real-time PCR equipment according to the manufacturer's instructions for use. **Note**: Before performing data analysis, select the preestablished analysis settings for each primers + probe system (i.e., baseline settings and threshold values) according to the 'RAID-CRC Symptomatic Technical Specifications' (this information can be found on the GoodGut website (www.goodgut.eu) or on the GoodGut-Test® platform (https://goodgut-test.eu/auth/login)).

Positive Control

The Positive Control is used to ensure the correct performance of the qPCR run. After setting the analysis settings the Ct obtained in the Positive Control must be comprised within the Ct range established in the 'RAID-CRC Symptomatic Technical Specifications'. When the Ct value of the Positive Control falls outside the accepted range values the results will not be valid. In this case, the sample analysis must be repeated.

No Template Control (NTC)

The No Template Control (NTC) is used to ensure that the reaction mix is not contaminated. After setting the analysis settings the Ct obtained in the NTC must be higher than the limit accepted values established in the 'RAID-CRC Symptomatic Technical Specifications'. When the NTC Ct value is lower than the limit accepted value, the results will not be valid since mix will probably be contaminated. In this case, the sample analysis must be repeated.

Note: 'RAID-CRC Symptomatic Technical Specifications' for your kit lot can be found on the GoodGut website www.goodgut.eu or on the GoodGut-Test® platform (https://goodgut-test.eu/auth/login).

2. **RAID-CRC Symptomatic diagnostic**.

Sample data

Before obtaining the diagnosis, the characteristics of the sample must be introduced into the platform for its proper identification. This information includes the sample's requirements to be suitable for the analysis, including the FIT value (obtained from any manufacturer) to be entered in μg haemoglobin per g of faeces. The information can be entered by following the User manual of the GoodGut-Test® platform (https://goodgut-test.eu/auth/login).

Results Report

To obtain the RAID-CRC Symptomatic diagnostic, the results obtain during the multiplex qPCR assay run must be introduced in the GoodGut-Test® platform (https://goodgut-test.eu/auth/login) following the User manual. The results must be uploaded to the platform using an excel file that must contain: sample identifier, dye, and the raw Ct data. The excel file can be downloaded on the platform following the User manual.

If an incident occurs, defined as any failure or problem that has occurred in this *In Vitro* Medical Device during its use or later and may have serious consequences for health, please contact the manufacturing laboratory: GoodGut e-mail: test@goodgut.eu.

RAID-CRC qPCR Kit
Variant: RAID-CRC Symptomatic qPCR Kit
UDI-DI: (01)08437023437025
Basic UDI-DI: 8437023437RAIDCRCKC

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