



## RAID-Dx

### Irritable Bowel Syndrome Diagnostic qPCR Kit

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**REF** Ref number:

DX-02-1024-01-LP (RAID-Dx qPCR Kit low profile)

DX-02-2024-01-HP (RAID-Dx qPCR Kit high profile)

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

#### Intended Purpose

The RAID-Dx qPCR Kit is intended for diagnosing irritable bowel syndrome and performing its differential diagnosis from inflammatory bowel disease, through the detection of specific microbial markers in DNA samples extracted from patients' faeces.

The *in vitro* RAID-Dx qPCR Kit diagnostic test is based on the qPCR analysis of a panel of faecal microorganisms that are indicators of both favourable and unfavourable intestinal health conditions. The panel is made up of 9 biomarkers representing different species, phylogroups, genera, and other taxa: *Faecalibacterium prausnitzii*, *F. prausnitzii* phylogroup I and *F. prausnitzii* phylogroup II, *Escherichia coli*, *Akkermansia muciniphila*, *Ruminococcus* sp., *Methanobrevibacter smithii*, Bacteroidetes, and Eubacteria. The qPCR Kit allows the amplification and quantification of characteristic gene fragments of the mentioned microorganisms. Results are given in a report both qualitatively and quantitatively. The product is not automated. The intended user is a laboratory professional.

#### Test principles

The RAID-Dx Kit is optimised for quantitative multiplexed PCR assays 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 the 5'



exonuclease activity of DNA polymerase. This enzyme cleaves the probes bound to the complementary DNA sequence during DNA amplification, 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 could be measured on real-time PCR platforms.

The RAID-Dx Kit requires three different qPCR assays for each sample to obtain a diagnostic. Therefore, RAID-Dx Kit includes 9 (3x3) 8-reactions tube strips, so that a total of 24x3 reactions can be performed. The master mix is delivered lyophilised together with the primers and probes in the tube strips preloaded in a stabilised format, which confers long term stability and avoids the need for cold chain. The product contains in each well all the components necessary for a qPCR assay with a final volume of 20 µL (including the DNA template). Three positive controls, one for each qPCR assay, are also provided lyophilised in separate tubes to check the correct performance of each qPCR assay.

## Requirements for RAID-Dx

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

- Faecal samples must come from subjects who present abdominal pain, depositional alterations, and/or meet Rome III or Rome IV criteria.
- Faecal samples must come from subjects over 18 years old.
- Faecal samples must be free of antibiotics from 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 acceptable.
- Faecal samples must be treated within the first 48 hours after sample collection. **Note:** upon arrival, the sample must be homogenised using a sterile spatula and then



proceed to the DNA extraction. If the DNA extraction cannot be done upon arrival, the sample can be frozen at -20°C.

## Kit Contents

Table 1. Components included in the RAID-Dx Kit.

RAID-Dx Kit (24x3 reactions   20 µL/reaction)	
3 x 8-reactions tube strips with GoodGut RAID-Dx Multiplex 1, including the following lyophilised components:	
	Multiplex Master Mix (Contains: enzymes, buffer, dNTP mix [dATP, dCTP, dGTP, dTTP], stabilizers)
	primer DX_1_f1
	primer DX_1_r1
	probe DX_1_FAM (contains the fluorochrome FAM and the quencher BHQ1)
	primer DX_1_f2
	primer DX_1_r2
	probe DX_1_HEX (contains the fluorochrome HEX and the quencher BHQ1)
	primer DX_1_f3
	primer DX_1_r3
	probe DX_1_ROX (contains the fluorochrome ROX and the quencher BHQ2)
3 x 8-reactions tube strips with GoodGut RAID-Dx Multiplex 2, including the following lyophilised components:	
	Multiplex Master Mix (Contains: enzymes, buffer, dNTP mix [dATP, dCTP, dGTP, dTTP], stabilizers)
	primer DX_2_f1
	primer DX_2_r1
	probe DX_2_FAM (contains the fluorochrome FAM and the quencher BHQ1)
	primer DX_2_f2
	primer DX_2_r2
	probe DX_2_HEX (contains the fluorochrome HEX and the quencher BHQ1)
	primer DX_2_f3
	primer DX_2_r3
	probe DX_2_ROX (contains the fluorochrome ROX and the quencher BHQ2)
3 x 8-reactions tube strips with GoodGut RAID-Dx Multiplex 3, including the following lyophilised components:	

**RAID-Dx Kit**

(24x3 reactions | 20 µL/reaction)

Multiplex Master Mix (Contains: enzymes, buffer, dNTP mix [dATP, dCTP, dGTP, dTTP], stabilizers)

primer DX\_3\_f1

primer DX\_3\_r1

probe DX\_3\_FAM (contains the fluorochrome FAM and the quencher BHQ1)

probe DX\_3\_HEX (contains the fluorochrome HEX and the quencher BHQ1)

primer DX\_3\_f2

primer DX\_3\_r2

probe DX\_3\_ROX (contains the fluorochrome ROX and the quencher BHQ2)

1 x GG1 Positive Control (contains a mixture of the qPCR amplification products of GoodGut RAID-Dx Multiplex 1)

1 x GG2 Positive Control (contains a mixture of the qPCR amplification products of GoodGut RAID-Dx Multiplex 2)

1 x GG3 Positive Control (contains a mixture of the qPCR amplification products of GoodGut RAID-Dx Multiplex 3)

Rehydration buffer (1.8 mL)

9 tear-off 8-cap strips

**Reagents, materials, and equipment not provided**

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

- Spatula
- 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 8.1 buffer (for positive controls resuspension)
- Microcentrifuge tubes
- Filter tips
- Vortex
- Centrifuge for 1.5 mL tubes
- Spin centrifuge
- Micropipettes (0.5 – 10 µL, 10 – 100 µL, and 100 – 1000 µL)
- Powder-free disposal gloves



## Transport and Storage Conditions

RAID-Dx qPCR Kits can be shipped and stored at 2-40°C until the expiration date stated on the label is reached. Keep all the 8-reactions tube strips stored in the corresponding aluminium pouch with silica gel provided. It is recommended to make some aliquots of the positive controls once resuspended to avoid undergoing more than 3 freeze/thaw cycles. Once the kit is in use, it can be stored at room temperature (2-40°C) and used until its expiration date stated on the label is reached.

## Safety Information

- For professional user *in vitro* use only.
- Do not use after 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.
- **Note:** There are no specific risks for the professional user, except those usual in an analysis laboratory



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

## Quality Control

Following GoodGut's ISO13485-certified Quality Management System, each lot of RAID-Dx qPCR Kit is tested against predetermined specifications to ensure activity, efficiency, and



sensitivity. The certificate of analysis (CoA) 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-Dx Kit.

Component	Description
GoodGut RAID-Dx Multiplex 1	3 x 8-reactions tube strips with Multiplex 1
Master Mix	Enzymes, buffer, dNTP mix [dATP, dCTP, dGTP, dTTP], stabilisers
Primers (forward and reverse)	Contains 3 primer sets purified using HPLC preloaded in the 8-reactions tube strips.
Probes	Contains 3 probes purified using HPLC preloaded in the 8-reactions tube strips.
GoodGut RAID-Dx Multiplex 2	3 x 8-reactions tube strips with Multiplex 2
Master Mix	Enzymes, buffer, dNTP mix [dATP, dCTP, dGTP, dTTP], stabilisers
Primers (forward and reverse)	Contains 3 primer sets purified using HPLC preloaded in the 8-reactions tube strips.
Probes	Contains 3 probes purified using HPLC preloaded in the 8-reactions tube strips.
GoodGut RAID-Dx Multiplex 3	3 x 8-reactions tube strips with Multiplex 3
Master Mix	Enzymes, buffer, dNTP mix [dATP, dCTP, dGTP, dTTP], stabilisers
Primers (forward and reverse)	Contains 2 primer sets purified using HPLC preloaded in the 8-reactions tube strips.
Probes	Contains 3 probes purified using HPLC preloaded in the 8-reactions tube strips.
Rehydration Buffer	Solution to reconstitute the stabilised product
GG1 Positive control GG2 Positive control GG3 Positive control	Each one contains the corresponding pool of 3 qPCR amplification products, which go through a quality control process including size verification by capillary electrophoresis and sequence identification by mass spectrometry.

## RAID-Dx qPCR Kit accessories

GoodGut-Test™ web platform (<https://goodgut-test.eu>) must be used to obtain the RAID-Dx diagnostic. The access to the platform is provided separately when the RAID-Dx qPCR product is acquired. The user manual is provided together with a DEMO of how the web platform works to professional laboratory users.



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
<b>SCALE</b>	125%	125%
<b>SCREEN RESOLUTION</b>	1920 x 1080	1920 x 1080
<b>SCREEN ORIENTATION</b>	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.

## RAID-Dx qPCR kit Protocol

This protocol must be followed for obtaining RAID-Dx results.

### **SAMPLE TREATMENT**

Faecal samples must be treated within the first 48 hours after sample collection. Upon arrival, the sample must be homogenised using a sterile spatula and then proceed to the DNA extraction. If the DNA extraction cannot be done upon arrival, the sample can be frozen at -20°C.

The results obtained using the RAID-Dx qPCR Kit are only reliable when a compatible DNA extraction kit and automated extractor are used (to check compatibility see Annex 1).

The sample information must be introduced into the GoodGut-Test™ web platform (<https://goodgut-test.eu/>) following the User manual, which is provided once you acquire the RAID-Dx qPCR Kit and also available on the Professional area of GoodGut website (<https://professionalarea.goodgut.eu/>). Sample information includes the requirements that must be fulfilled before being analysed and a sample code to properly follow its traceability.



## qPCR PROTOCOL

The preloaded master mix and primers/probe, as well as the parameters (temperatures (annealing), cycles number, and step times), have been optimised for an optimal yield and specificity of the multiplex assay.

Before starting, resuspend the positive controls with 25  $\mu$ L of Tris-HCl pH 8.1 buffer. **Note:** For proper resuspension after the Rehydration buffer addition, incubate the tubes at room temperature for 1 hour or overnight at 4°C. Once resuspended they should be stored at -20°C in a constant-temperature freezer and protected from light.

To obtain the diagnostic, three multiplex qPCR assays for each sample are required: GoodGut RAID-Dx Multiplex 1, GoodGut RAID-Dx Multiplex 2, and GoodGut RAID-Dx Multiplex 3. For each different multiplex assay (Multiplex 1, Multiplex 2, and Multiplex 3) perform steps 1 to 4 separately using the indicated 8-reactions tube strips (i.e., steps 1 to 4 will be repeated three times, one for each multiplex qPCR assay).

1. Determine and separate the number of tubes (from the 8-reaction tube strips) for the required reactions including samples and the two indicated controls for the qPCR multiplex assay to be performed (Multiplex 1, Multiplex 2, or Multiplex 3). One positive control and no-template control (NTC) should be included in each qPCR assay. **Note:** Each qPCR has its own positive control.
2. Reconstitute the number of wells you need. Peel off protective aluminium seal from strips and add 18  $\mu$ L of Rehydration Buffer in each well.
3. Add 2  $\mu$ L of the DNA samples to the individual qPCR tubes that contain the reaction mix. Add also 2  $\mu$ L of the positive control specified for the qPCR multiplex assay which is being performed (GG1 positive control for Multiplex 1, GG2 positive control for Multiplex 2, or GG3 positive control for Multiplex 3) to the tube reserved for this control and leave a tube only with the reaction mix as a no template control (NTC). Close the strips with the optical caps provided, vortex the strips vigorously (5 seconds), and perform a spin to ensure that the reaction mix is at the bottom of the tube without bubbles.



- Repeat steps 1 to 3 for the other two qPCR multiplex assays to complete the RAID-Dx analysis.
- Load the strips in the thermocycler (to check compatibility see Annex 2).
- Program your thermocycler according to Table 4. **Note:** Select the specific channels (targets) to fluorogenic data acquisition that can be performed during the combined annealing/extension step: FAM, HEX, and ROX for the three RAID-Dx multiplex qPCR assays.

Table 4. Thermal cycling protocol for RAID-Dx multiplex qPCR assay.

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

- Start the run. **Note:** all the samples and controls of the same kind of qPCR assay (Multiplex 1, or Multiplex 2, or Multiplex 3) must be analysed in the same qPCR run. In case of using more than one thermocycler and/or qPCR instruments for the analysis of the same sample (i.e., Multiplex 1 analysed in thermocycler 1 and Multiplex 2 analysed in thermocycler 2), make sure that the same model is being used.

## ANALYSIS AND INTERPRETATION OF THE RESULTS

- 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-Dx Technical Specifications' (this information is provided once you acquire the RAID-Dx qPCR Kit and on the Professional area of GoodGut website <https://professionalarea.goodgut.eu/>).



2. To obtain the RAID-Dx diagnostic, the results obtained during each multiplex qPCR assay run (including positive and negative controls) must be introduced in the GoodGut-Test™ web platform (<https://goodgut-test.eu/>) following the User manual. The results must be uploaded to the platform using its specific excel file that must contain sample code, dye, and the raw Ct data (Cq). The excel files template can be downloaded in the platform following the User manual.

### 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-Dx Technical Specifications'. When the Ct value of the Positive Control falls outside the accepted range values, the results will not be reliable. The GoodGut-Test™ platform informs if the positive controls are accepted or rejected. If the positive controls are rejected, 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-Dx Technical Specifications'. When the NTC Ct value is lower than the limit accepted value, the results will not be reliable since mix will probably be contaminated. The GoodGut-Test™ web platform informs if the NTCs are accepted or rejected. If the NTCs are rejected, the sample analysis must be repeated.

**Note:** The specific 'RAID-Dx Technical Specifications' for your specific lot and the User Manual are provided separately when acquiring the kit and are also found on the Professional area of GoodGut website <https://professionalarea.goodgut.eu/>.



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

### Symbol description

	Reference or catalogue number
	Amount of liquid or reagent in the vial or bottle
	Read the instructions for use

RAID-Dx qPCR Kit  
Irritable Bowel Syndrome Diagnostic qPCR Kit  
GoodGut SRN: ES-MF-000000229

Basic UDI-DI:  
8437023437RAIDDX9Y

UDI of the variants:  
- UDI (Low Profile): (01)08437023437032  
- UDI (High Profile): (01)08437023437049

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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-Dx are the following:

### DNeasy Powersoil Pro DNA extraction kit from Qiagen (**manual extraction**)

- Kit Reference: 47014, QIAGEN
- Proceed following the manufacturer's instructions. **Note:** instead of using 250- 500 mg of soil in Step 1, weigh around 50 mg of faeces.

### QIAcube from Qiagen (**automated extractor**)

- Use the DNeasy Powersoil Pro DNA extraction kit from Qiagen with the automated extractor QIAcube Connect from QIAGEN.
- Proceed following the manufacturer's instructions. **Note:** instead of using 250- 500 mg of soil in Step 1, weigh around 50 mg of faeces.



## ANNEX 2: COMPATIBILITY OF THE REAL TIME PCR EQUIPMENT

Low profile strips can be used 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-Dx 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-Dx qPCR Kit'.

**Note:** The specific 'RAID-Dx 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/>.