RAID-Dx
Irritable Bowel Syndrome Diagnostic qPCR Kit

General description

The RAID-Dx Kit is optimized for quantitative PCR 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 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 reactions each one, 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 stabilized 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.

Intended Use

The RAID-Dx 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.

Requirements for RAID-Dx

This RAID-Dx kit has been optimized 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 IV criteria.
- Faecal samples must be free of antibiotics from the month prior to deposition.
- Faecal samples are not acceptable from pregnant women.

- Faecal samples must be treated within the first 48 hours after sample collection. **Note**: upon arrival, the sample must be homogenized 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 -20ºC.
- The results obtained using the RAID-Dx kit are only reliable when the DNeasy Powersoil Pro DNA extraction Kit from Qiagen is used. Proceed following the manufacturer's instructions. **Note**: instead of using 250-500 mg of soil in Step 1, weigh around 50 mg of faeces.

Kit Contents

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

RAID-Dx Kit (24x3 | 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)

RAID-Dx Kit (24x3 | 20 µL/reaction)	
3 x 8-reactions tube strips with GoodGut RAID-Dx Multiplex 3, including the following lyophilised components:	
	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	
9 tear-off 8-cap strips	

Reagents, materials, and equipment not supplied

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 kit.

- Spatula
- Thermocycler and/or Real-Time PCR instrument (to check compatibility see 'RAID-Dx 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 8.1 Buffer (for positive controls resuspension)
- GoodGut-Test® platform for RAID-Dx diagnosis (https://goodgut-test.eu/auth/login)
- 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 Kits can be shipped and stored at 2-40ºC until the expiration date stated on the label. 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 freezing/thawing cycles.

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.

⚠	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 Kit is tested against predetermined specifications to ensure activity, efficiency, and sensitivity. The certificate of analysis (CoA) can be found on the GoodGut website: www.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], stabilizers
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], stabilizers
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], stabilizers
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 stabilized 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.

qPCR Protocol

This protocol must be followed for obtaining RAID-Dx results. The preloaded master mix and primers/probe, as well as the parameters (temperatures (annealing), cycles number, and step times), have been optimized for an optimal yield and specificity of the multiplex assay.

Before starting, resuspend the positive controls with 25 µL 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 4ºC. Once resuspended they should be stored at -20ºC 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-reactions 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 and no-template control (NTC) should be included in each qPCR assay. **Note**: Each qPCR has its positive control.
2. Reconstitute the number of wells you need. Peel off protective aluminium seal from strips and add 18 µL of Rehydration Buffer in each well.
3. 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 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.
4. Repeat steps 1 to 3 for the other two qPCR multiplex assays to complete the RAID-Dx analysis.
5. Load the strips in the thermocycler.
6. Program your thermocycler according to Table 3. **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 Dx multiplex assays.

Table 3. Thermal cycling protocol for RAID-Dx 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. 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

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-Dx 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-Dx Technical Specifications'. When the Ct value of the Positive Control falls outside the accepted range values the results will not be reliable. 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-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. In this case, the sample analysis must be repeated.

Note: The specific 'RAID-Dx 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-Dx 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 and it 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-Dx diagnostic, the results obtained 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 in 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 S.L.U. e-mail: test@goodgut.eu.

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RAID-Dx qPCR Kit
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Basic UDI-DI: 8437023437RAIDDX9Y

Variants UDI:
- UDI (Low Profile): (01)08437023437032
- UDI (High Profile): (01)08437023437049

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