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RAID-Dx qPCR Kit

Irritable Bowel Syndrome Diagnostic qPCR Kit

INSTRUCTIONS FOR USE

Ref number: REF

- *RAID-Dx qPCR Kit (low profile): DX-02-1024-01-LP*
- *RAID-Dx qPCR Kit (high profile): DX-02-2024-01-HP*

The **RAID-Dx qPCR Kit** is an *in vitro* medical device (diagnostic 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.

This test can diagnose irritable bowel syndrome and making its differential diagnosis with inflammatory bowel diseases with a sensitivity and specificity greater than 85% (Table 1).

Table 1. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) values of the RAID-Dx qPCR Kit for the diagnosis of irritable bowel syndrome (IBS) and its differential diagnosis with inflammatory bowel diseases (IBS vs. IBD).

	IBS	IBS vs IBD
Sensitivity (%)	91.0	94.8
Specificity (%)	86.1	87.6
PPV (%)	80.0	91.0
NPV (%)	94.7	93.6

The product is not automated. The intended user is a laboratory professional.

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Test principles

The RAID-Dx Kit is optimised for quantitative multiplexed PCR assays using specific primers and fluorescent-labelled probes in bacterial DNA from faecal samples. 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:

Target population of the test:

- 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 -20ºC.*

These requirements are necessary so that the test results fall within the established reference ranges for the diagnosis of irritable bowel syndrome using the RAID-Dx qPCR kit.

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Kit Contents

Table 2. Components included in the RAID-Dx Kit.

Reagent/Material	Description	Concentration range	Colour	Amount
Multiplex 1 **8-well strips**	Lyoprotectors and Stabilizers	±6 g/100 mL*	White (Opaque)	3 x 8-well strips
	Nucleotide triphosphate (dNTPs)	±1 mM*		
	3 Primers and probes sets	0.2-1 nMol/µL*		
	Enzymes	10-100 U/rxn*		
Multiplex 2 **8-well strips**	Lyoprotectors and Stabilizers	±6 g/100 mL*	White (Opaque)	3 x 8-well strips
	Nucleotide triphosphate (dNTPs)	±1 mM*		
	3 Primers and probes sets	0.2-1 nMol/µL*		
	Enzymes	10-100 U/rxn*		
Multiplex 3 **8-well strips**	Lyoprotectors and Stabilizers	±6 g/100 mL*	White (Opaque)	3 x 8-well strips
	Nucleotide triphosphate (dNTPs)	±1 mM*		
	2 Primers and probes sets	0.2-1 nMol/µL*		
	Enzymes	10-100 U/rxn*		
Positive control GG1	Synthetic lyophilized DNA	$1.9x10^4$ copies/µL*	Red	1 vial
Positive control GG2	Synthetic lyophilized DNA	$1.9x10^4$ copies/µL*	Red	1 vial
Positive control GG3	Synthetic lyophilized DNA	$1.9x10^4$ copies/µL*	Red	1 vial
Rehydration Buffer	Saline solution mixture	±13 mM	Blue	1 vial x 1.8 mL
	Buffer (TRIS, pH)	±67 mM		
8-cap strips	Tear-off 8-cap strips	NA	Transparent	9 x 8-cap strips

** For component in stabilized format, the concentration range means after rehydration, NA: Not Applicable*

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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 2-40ºC 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 a few aliquots of the positive controls after resuspension to avoid more than 3 freeze/thaw cycles and keep them at -20°C. Once the kit is in use, it can be stored at room temperature (2-40°C) and used until the expiry date indicated on the label (except for the resuspended positive control which should be stored at -20°C). It should be noted that, although the kit can be stored in the temperature range described. It should be noted that, although the kit can be stored within the temperature range described. It is recommended to store at an average temperature of 25ºC ± 5ºC.

Stability in use

Storage conditions: between 2ºC and 40ºC, *see transport and storage conditions section.*

Shelf life after opening the primary container/packaging: what the packaging indicates. After opening the primary container, tests demonstrate the optimal stability of the product (all its reagents on the strips) until the expiration date indicated on the packaging.

The results obtained showed optimal performance of the rehydrated product within the working day (about 8 hours), however, we recommend that once the strips are rehydrated, they are not stored. The experiment must be carried out immediately.

Freezing and thawing cycles aliquots positive controls: see transport and storage conditions.

Master mix and positive controls: The mastermix, once resuspended, can be at least 24 hours at 4ºC. The positive controls, once resuspended, can be stored for at least 12 months at -20ºC.”

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Safety Information

- For professional user 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 precautions in an analysis laboratory.*

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

Information on interfering substances

See RAID-Dx analysis requirements section, page 2.

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/.

Limitations of Use:

The reagents in this kit are designed to work entirely with this qPCR kit. **It is not recommended for use with other tests.** These reagents are suitable for the following instruments, *see annex 2.*
So far, the product has not been found to contain any other components that may influence the measurements.

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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
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 the correct performance of the qPCR RAID-Dx kit, positive controls of known concentration are included. Positive controls must be added for each run of multiplex qPCR assays (as detailed in the qPCR RAID-Dx kit protocol section, page 8). In addition, a control without template DNA or negative control (NTC) is required to ensure that multiplex qPCR assays run are not contaminated.

Positive Control

Since the methodology used is a multiplex qPCR assay where 3 biomarkers are analysed at the same time, the positive control is also a pool of the target markers analysed in each qPCR assay. A specific tolerance range is defined for each batch of positive control. Tolerance ranges are obtained by analysing 5 different runs of a randomly chosen positive control from each batch. The tolerance range for each marker is calculated by averaging the results ± 10%.

Once the analysis and interpretation of the results is carried out, the Ct value obtained for the positive control must fall within the established Ct range per batch. When the Ct value of the positive control is outside the accepted range the results are not reliable. The GoodGut-Test™ web platform reports whether positive controls are accepted or rejected. If the positive controls are rejected, the sample analysis must be repeated.

The range of positive control tolerances are available to all customers in the batch-specific RAID-Dx qPCR kit technical specifications provided to all customers at the time of 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 (NTC) is used to ensure that the reaction mix is not contaminated. A specific tolerance range is defined for each batch of the RAID-Dx qPCR kit. Tolerance ranges are obtained by analysing 5 different runs of a randomly chosen kit from each batch. The tolerance range of the NTC each marker is calculated by averaging the results - 10%.

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Once the analysis and interpretation of the results is carried out, the Ct value obtained in the NTC must be higher than the established limit value per batch. When the Ct value of the NTC is outside the accepted range the results are not reliable. The GoodGut-Test™ web platform inform whether the NTCs are accepted or rejected. If the NTCs are rejected, the sample analysis must be repeated.

The tolerance range of the NTCs is available to all customers in the batch-specific RAID-Dx qPCR kit technical specifications provided to all customers at the time of purchase. The technical specifications can also be found in the professional area of the GoodGut website: https://professionalarea.goodgut.eu/.

RAID-Dx qPCR kit Protocol

This protocol must be followed to obtain 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 -20ºC.

  The results obtained using the RAID-Dx qPCR Kit are only reliable when a compatible DNA extraction kit and/or 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 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 (See transport and storage conditions).*

  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 positive control.*
  2. Reconstitute the number of wells you need. Peel off the protective aluminium seal from the 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 (to check compatibility see *Annex 2*).
6. 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 (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 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.
3. The platform applies a mathematical algorithm considering the reference intervals of populations with irritable bowel syndrome and generates a diagnostic report. The diagnostic report has one part:

   a. **Qualitative:**

      i. Irritable bowel syndrome positive

      ii. Irritable bowel syndrome non-compliant.

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b. **Quantitative:** Colour traffic light showing the characteristics of the analysed markers:

   i. Green: the analysed marker is within the reference range considered to be healthy bowel.

   ii. Orange: the analysed marker is at the limit of the reference interval.

   iii. Red: the analysed marker is outside the reference interval considered a deviation.

4. The final report should be interpreted by a specialist.

***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/.*

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In the event of an incident, defined as any malfunction or problem that has occurred with this In Vitro Medical Device (In Vitro Medical Device), during or after use, and which may have serious health consequences, please contact the manufacturing laboratory: GoodGut S.L.U. e-mail: test@goodgut.eu and/or the competent authority where the user and/or patient is established.

Symbol description

REF	Reference or catalogue number
VOL	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
Basic UDI-DI: 8437023437RAIDDX9

GOODGUT
Enhancing digestive health

GOODGUT, SLU
CIF/NIF: B55206916
GoodGut SRN: ES-MF-000000229
C/Pic de Peguera, 11, 17003 Girona - Cataluña, España.
Teléfono: +34 972 18 32 20.
E-mail: info@goodgut.eu
www.goodgut.eu

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

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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 40 mg of faeces.*

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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'.

High profile strips can be used in all qPCRs thermocyclers equipped with a high-profile block listed below.

Quantstudio 5 (Applied Biosystems™)

- 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 (e.g., baseline settings and threshold values) according to the *'Technical specifications of the qPCR RAID-Dx 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/.*