# RIDA®GENE Faecalibacterium prausnitzii

REF PG0155

R-Biopharm AG, An der neuen Bergstraße 17, 64297 Darmstadt, Germany
Phone: +49 (0) 61 51 81 02-0 / Fax: +49 (0) 61 51 81 02-20

## 1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Faecalibacterium prausnitzii is a multiplex real-time PCR for the direct qualitative and quantitative detection of *Faecalibacterium prausnitzii* DNA in human stool samples.

## 2. Summary and explanation of the test

*Faecalibacterium prausnitzii* is a gram-positive, strictly anaerobic, commensal bacterium and belongs to the family of *Clostridiacea*. *F. prausnitzii* is one of the most important bacteria in the human gut flora and makes up to 5 - 10 % of the total number of bacteria detected in stool samples from healthy humans.[1] *F. prausnitzii* localization seemed to depend upon anaerobic conditions only found in the lower gut.[2] In early infancy the number of *F. prausnitzii* is very low and increases after the establishment of primocolonizing bacteria.[2] They are strictly anaerobic and thus difficult to grow in culture. Consequently, this explains why the mechanisms of its protection potential are still poorly understood. *F. prausnitzii* produces butyric acid derivates e.g. butyrate, which are essential for the intestinal activity. Butyrates play an important role for the metabolism in the colon. They serve as energy sources for the colonocytes and have anti-inflammatory effects and maintain the activity of bacterial enzymes and protect the digestive system against intestinal pathogens.[3] Changes in the number of *F. prausnitzii* are an indication for dysbiosis in the human gut flora. A significant reduction of *F. prausnitzii* is detected by patients with diabetes and chronic inflammatory intestinal disorders (Morbus Crohn, Colitis ulcerosa, Irritable Bowel Syndrome [IBS]). For the latter case, the intestinal barrier is disrupted or rather the intestinal wall gets more permeable. This leads to an unregulated mass exchange and causes diarrheal diseases and inflammatory reactions.

## 3. Test principle

RIDA®GENE Faecalibacterium prausnitzii is a multiplex real-time PCR for the direct, qualitative and quantitative detection of *Faecalibacterium prausnitzii* in human stool samples.
After DNA isolation, amplification of the gene fragment (if present) specific for *Faecalibacterium prausnitzii* (16S-rRNA) occurs. The amplified targets are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step, the Taq-Polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal, which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. With the standards, Standard A, Standard B and Standard C, included in the kit, it is possible to quantify the results. The identified DNA amount in the sample (copies/reaction) is converted into the concentration unit cells / g stool with a correction factor (K, see also table 12). The RIDA®GENE Faecalibacterium prausnitzii multiplex real-time PCR kit contains an Internal Control DNA (ICD) that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient.

## 4. Reagents provided

**Tab. 1:** Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

| Kit Code | Reagent | Amount | | Lid Color |
|---|---|---|---|---|
| **1** | Reaction Mix | 2x | 1050 µl | yellow |
| **2** | Taq-Polymerase | 1x | 80 µl | red |
| **D** | Internal Control DNA | 2x | 1700 µl | orange |
| **N** | No Template Control | 1x | 450 µl | white |
| **P** | Positive Control | 1x | 200 µl | blue |
| **10^2** | Standard A | 1x | 100 µl | dark blue |
| **10^4** | Standard B | 1x | 100 µl | dark blue |
| **10^6** | Standard C | 1x | 100 µl | dark blue |

## 5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used unopened until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 20 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all the reagents should be stored cold in an appropriate way (2 - 8 °C).

## 6. Additional necessary reagents and necessary equipment

The RIDA®GENE Faecalibacterium prausnitzii multiplex real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

**Tab. 2**: Necessary equipment

| **Extraction platform** | |
|---|---|
| R-Biopharm | RIDA® Xtract |
| Promega | Maxwell® RSC |
| **Real-time PCR instrument** | |
| Roche | LightCycler® 2.0, LightCycler® 480II |
| Agilent Technologies | Mx3005P |
| Applied Biosystems | ABI 7500 |
| Bio-Rad | CFX96™ |
| QIAGEN | Rotor-Gene Q |

**Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).**

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit II (PG0002) for use with the LightCycler® 2.0
- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (nuclease-free)

## 7. Precautions for users

For *in-vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use.
Please refer to the relevant national regulations for disposal.
For more details see Safety Data Sheets (SDS) at www.r-biopharm.com

## 8. Collection and storage of samples

### 8.1 Sample preparation from stool samples

For DNA isolation of human stool samples, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.
We recommend to dilute the stool samples before extraction 1:3 with water. Vortex the diluted stool sample intensely and centrifuge at 1000 x g for 30 sec. Use from the supernatant the appropriate volume according to the manufacturer's instruction.

The RIDA®GENE Faecalibacterium prausnitzii assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab.4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture

and must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

## 9. Test procedure

### 9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and one negative control must be included in each assay run.

We recommend to calculate an additional volume of 10 % to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control, the Internal Control DNA and Standard A, Standard B and Standard C before using. Keep reagents appropriately cold during working step (2 - 8 °C).

**Tab. 3**: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

| Kit code | Master-Mix components | Volume per reaction | 10 reactions (10 % extra) |
|---|---|---|---|
| **1** | Reaction Mix | 19.3 µl | 212.3 µl |
| **2** | Taq-Polymerase | 0.7 µl | 7.7 µl |
| | **Total** | **20 µl** | **220 µl** |

Mix the components of the Master-Mix gently and briefly spin down.

**Tab. 4**: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

| Kit code | Master-Mix components | Volume per reaction | 10 reactions (10 % extra) |
|---|---|---|---|
| **1** | Reaction Mix | 19.3 µl | 212.3 µl |
| **2** | Taq-Polymerase | 0.7 µl | 7.7 µl |
| **D** | Internal Control DNA | 1.0 µl | 11 µl |
| | **Total** | **21.0 µl** | **231.0 µl** |

Mix the components of the Master-Mix gently and briefly spin down.

### 9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

**Negative control:** Add 5 µl No Template Control to the pre-pipetted Master-Mix.

**Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the negative control.**

**Sample:** Add 5 µl eluate to the pre-pipetted Master-Mix.

**Positive control:** Add 5 µl Positive Control to the pre-pipetted Master-Mix.

**Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the positive control.**

**Standard (A, B, C):** Add 5 µl Standard (A, B, C) to the pre-pipetted Master-Mix.

**Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the standards.**

**Note: Using the following cyclers requires to include a standard curve in each run: ABI 7500 (Applied Biosystems) and CFX96™ (Bio-Rad).**

**For all other cyclers, only one sample of the standard curve (Standard B) has to be included in the experimental set-up as calibrator for each new real-time PCR run. Here, the application of a standard curve is only required to run once per lot number.**

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument set-up (see Tab. 5, Tab. 6, Tab. 7, Tab. 8).

## 9.3 PCR instrument set-up

### 9.3.1 DNA real-time PCR profile

**Tab. 5**: DNA real-time PCR profile for LightCycler® series and Rotor-Gene Q

| | |
|---|---|
| Initial Denaturation | 1 min, 95 °C |
| Cycles | 45 Cycles |
| PCR Denaturation<br>Annealing/Extension | 10 sec, 95 °C<br>15 sec, 60 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

**Note: Annealing and extension occur in the same step.**

**Tab. 6**: DNA real-time PCR profile for Mx3005P, ABI7500, CFX96™

| | |
|---|---|
| Initial Denaturation | 1 min, 95 °C |
| Cycles | 45 Cycles |
| PCR Denaturation<br>Annealing/Extension | 15 sec, 95 °C<br>30 sec, 60 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

**Note: Annealing and extension occur in the same step.**

**Note: The total copy number per reaction of Standard A, Standard B and Standard C has to be typed in into the Setup File of the software program of the respective real-time PCR cycler. A total volume of 5 µl DNA is used resulting in following concentrations:**

**Standard A: 5 x $10^2$ copies/reaction**

**Standard B: 5 x $10^4$ copies/reaction**

**Standard C: 5 x $10^6$ copies/reaction**

**Note: The standard curve can be saved on the real-time PCR cycler for each parameter. Apart from the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cycler, the standard curve is only required to run once per lot number. Using the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cycler requires to include a standard curve in each run. For all other cyclers, only one sample of the standard curve (Standard B) has to be included in the experimental set-up as calibrator for each new real-time PCR run.**

### 9.3.2 Universal real-time PCR profile

**Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RNA real-time PCR assays in one run.**

**Tab. 7**: Universal real-time PCR profile for LightCycler® series

| | |
|---|---|
| Reverse Transcription | 10 min, 58 °C |
| Initial Denaturation | 1 min, 95 °C |
| Cycles | 45 Cycles |
| PCR Denaturation<br>Annealing/Extension | 10 sec, 95 °C<br>15 sec, 60 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

**Note: Annealing and Extension occur in the same step.**

**Tab. 8**: Universal real-time PCR profile for Mx3005P, ABI7500, CFX96™ and Rotor-Gene Q

| | |
|---|---|
| Reverse Transcription | 10 min, 58 °C |
| Initial Denaturation | 1 min, 95 °C |
| Cycles | 45 Cycles |
| PCR Denaturation<br>Annealing/Extension | 15 sec, 95 °C<br>30 sec, 60 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

**Note: Annealing and Extension occur in the same step.**

**Note: The total copy number per reaction of Standard A, Standard B and Standard C has to be typed in into the Setup File of the software program of the respective real-time PCR cycler. A total volume of 5 µl DNA is used resulting in following concentrations:**

**Standard A: 5 x $10^2$ copies/reaction**

**Standard B: 5 x $10^4$ copies/reaction**

**Standard C: 5 x $10^6$ copies/reaction**

**Note: The standard curve can be saved on the real-time PCR cycler for each parameter. Apart from the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cycler, the standard curve is only required to run once per lot number. Using the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cycler requires to include a standard curve in each run.**

**For all other cyclers, only one sample of the standard curve (Standard B) has to be included in the experimental set-up as calibrator for each new real-time PCR run.**

## 9.4 Detection channel set-up

**Tab. 9**: Selection of appropriate detection channels

| Real-time PCR instrument | Detection | Detection channel | Note |
|---|---|---|---|
| **Roche LightCycler® 2.0** | *Faecalibacterium prausnitzii* | 530 | **RIDA®GENE Color Compensation Kit II (PG0002) is required** |
| | ICD | 560 | |
| **Roche LightCycler® 480II** | *Faecalibacterium prausnitzii* | 465/510 | **RIDA®GENE Color Compensation Kit IV (PG0004) is required** |
| | ICD | 533/580 | |
| **Agilent Technologies Mx3005P** | *Faecalibacterium prausnitzii* | FAM | **Check that reference dye is none** |
| | ICD | HEX | |
| **ABI 7500** | *Faecalibacterium prausnitzii* | FAM | **Check that passive reference option ROX is none** |
| | ICD | VIC | |
| **Bio-Rad CFX96™** | *Faecalibacterium prausnitzii* | FAM | **-** |
| | ICD | VIC | |
| **Qiagen Rotor-Gene Q** | *Faecalibacterium prausnitzii* | Green | **The gain settings have to be set to 5, according to the default settings** |
| | ICD | Yellow | |

## 10. Quality control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer's instructions. Positive control and negative control have to show correct results (see Table 10, Fig. 1) in order to determine a valid run.

The Positive Control has a concentration of $10^3$ copies/µl. In each PCR run it is used in a total amount of 5 x $10^3$ copies.

**Tab. 10**: For a valid run, the following conditions must be met:

| Sample | Assay result | ICD Ct | Target Ct |
|---|---|---|---|
| Positive control | Positive | NA *[1] | See Quality Assurance Certificate |
| Negative control | Negative | Ct > 20 | Not detectable |

*[1] *No Ct value is required for the ICD to make a positive call for the positive control.*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid, prepare all new reactions including the controls.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

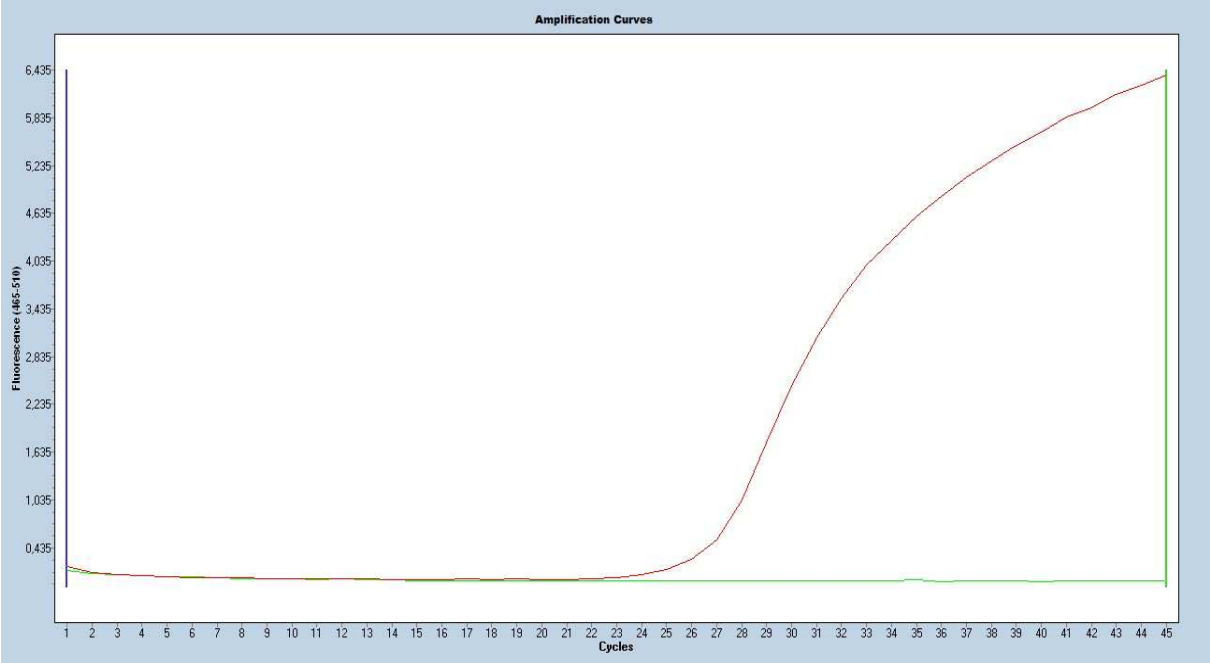


**Fig. 1**: Correct run of the positive control (red) and negative control (green) (*Faecalibacterium prausnitzii*) on the LightCycler® 480II

## 10.1 Validity of quantitative detection

For the validity of a quantitative diagnostic test run, all control conditions of a valid qualitative diagnostic test run must be met. Furthermore, for accurate quantification results a valid standard curve has to be generated. For a valid quantitative diagnostic test run, the following control parameter values of the standard curve should be achieved.

| | Control parameter | Valid value |
|---|---|---|
| **Roche LightCycler® 2.0** | Efficiency | 1,9 – 2,1 |
| **Roche LightCycler® 480II** | Efficiency | 1,9 – 2,1 |
| | Slope | -3,1 – -3,6 |
| **Agilent Techn. Mx3005P** | Rsq | > 0,98 |
| | Slope | -3,1 – -3,6 |
| **ABI 7500** | R^2 | > 0,98 |
| | Slope | -3,1 – -3,6 |
| **Bio-Rad CFX96™** | R^2 | > 0,98 |
| | Slope | -3,1 - -3,6 |
| **Qiagen Rotor-Gene Q** | R^2 | > 0,98 |
| | M | 3,1 – -3,6 |

## 11. Result interpretation

The result interpretation is done according to Table 11.

**Tab. 11**: Sample interpretation

| **Target genes** | | |
|---|---|---|
| ***Faecalibacterium prausnitzii*** | **ICD** | **Result** |
| **positive** | **positive / negative** | ***Faecalibacterium prausnitzii* detected** |
| negative* | **positive** | **Target genes not detected*** |
| negative | **negative** | **Invalid** |

*Faecalibacterium prausnitzii* is detected, if the sample DNA and the Internal Control DNA show an amplification signal in the detection system.

*Faecalibacterium prausnitzii* is also detected, if the sample DNA shows an amplification signal but none for the Internal Control DNA in the detection system. The detection of the internal amplification control is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control DNA.

*Faecalibacterium prausnitzii* is not detected, if the sample DNA shows no amplification signal, but an amplification signal for Internal Control DNA in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control DNA.

A sample is invalid, if the sample DNA and Internal Control DNA show no amplification signal in the detection system. The sample contains a PCR inhibitor. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

***Note:** **A negative result for *Faecalibacterium prausnitzii* DNA is unlikely since this bacterial group belongs to the human commensal bacteria. If a negative result occurs for *Faecalibacterium prausnitzii* DNA, it is likely that (upon use of the ICD as inhibition control) the sample extraction was not successful. If a negative result occurs for *Faecalibacterium prausnitzii* DNA, it is recommended to improve isolation and purification of the sample and repeat amplification.**

### 11.1 Quantification of samples

To quantify *Faecalibacterium prausnitzii* positive samples, a standard curve with the Standard A, Standard B and Standard C has to be performed separately. The standard curve measurement has to be saved separately. However, the same standard curve measurement can be used in all runs with products from the same lot number by importing the saved experiment.

**Note: This is not valid for the following cyclers: ABI 7500 (Applied Biosystems), and CFX96™ (Bio-Rad). Here, a standard curve has to be measured with each run. For all other cyclers, one sample of the standard curve (Standard B) has to be included in the experimental set-up as calibrator for each new real-time PCR run.**

To quantify *Faecalibacterium prausnitzii* positive samples, all standard samples (A, B and C), the positive and the negative control as well as the unknown samples to be quantified, have to be selected and analyzed according to the instructions of the cycler manufacturer.

With the quantitative RIDA®GENE Faecalibacterium prausnitzii multiplex real-time PCR the amount of DNA in copies/reaction of the parameter is calculated. The conversion into the concentration unit cells/g stool sample is done with a correction factor K and takes into account the dilutions of the extraction procedure (dependent on the extraction kit used) and the PCR set-up as well as the number of target sequences in the whole genome.

The conversion of the result of the quantitative RIDA®GENE Faecalibacterium prausnitzii multiplex real-time PCR in cells/g stool is calculated with following formula:

**C [cells/g stool] = c [copies/reaction] x K**

| | |
|---|---|
| C [cells/g stool] | - bacterial concentration of sample in cells/g stool |
| c [copies/reaction] | - DNA concentration in PCR reaction (result of quantitative PCR) |
| K | - correction factor |

For the calculation of the correction factor, following information has to be considered:
- Sample dilution
- Starting volume of sample for DNA extraction
- DNA extract from total eluate used for PCR reaction
- Number of target sequence in the whole genome

**Tab. 12**: Example calculation of correction factor using Maxwell® RSC (Promega) for sample preparation of a 1:3 diluted sample

| Description | Factor |
|---|---|
| Sample dilution 1:3 before extraction | x 3 |
| 300 µl sample for extraction* | x 3.33 |
| 5 µl DNA extract into PCR reaction** | x 20 |
| Target sequence contained 5x in total *Faecalibacterium prausnitzii* genome | x 0.2 (*Faecalibacterium prausnitzii*) |
| Correction factor K for *Faecalibacterium prausnitzii**** | 0.40 x $10^2$ |

* Result corresponds to 1 g stool
** Corresponding to a total eluate of 100 µl (= 1/20)
*** This value can be saved in the real-time PCR instrument

**Note: For further information on quantification of please contact mdx@r-biopharm.de.**

## 12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for human stool samples.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Faecalibacterium prausnitzii assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target genes (16S-rRNA).

## 13. Performance characteristics

### 13.1 Analytical sensitivity

The RIDA®GENE Faecalibacterium prausnitzii multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction for *Faecalibacterium prausnitzii*.

The following figure 2 shows a dilution series of *Faecalibacterium prausnitzii* ($10^6$ - $10^2$ DNA copies per µl) on the LightCycler® 480II.

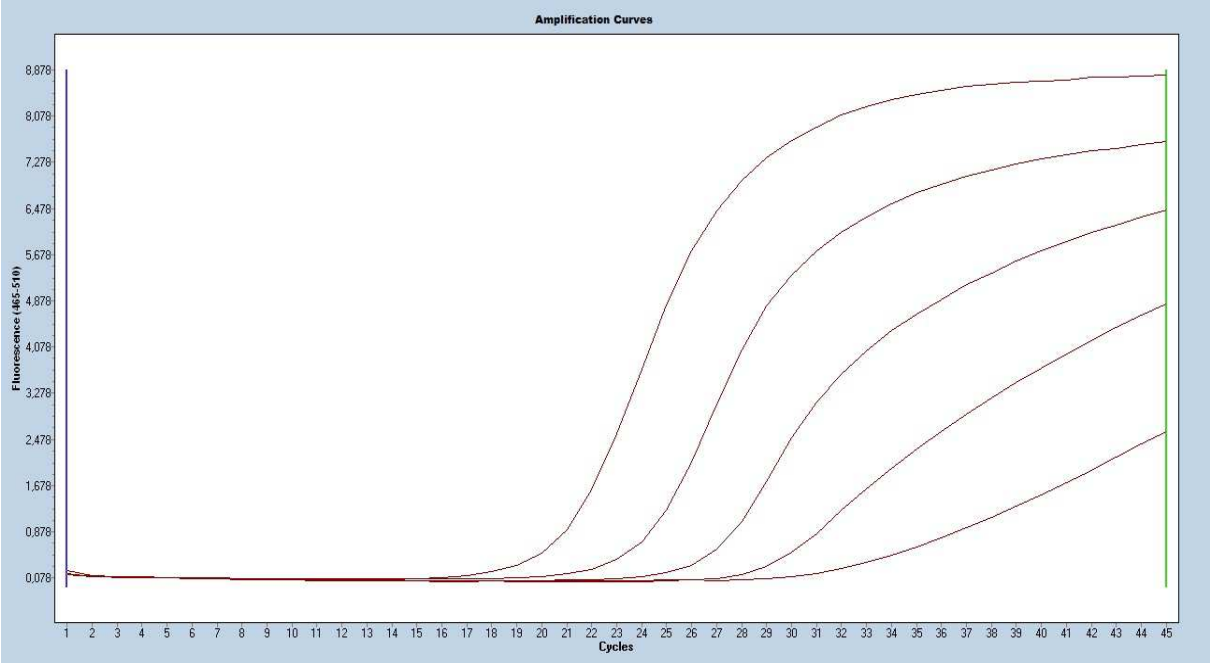


**Fig. 2**: Dilution series *Faecalibacterium prausnitzii* ($10^6$ – $10^2$ DNA copies per µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration.

## 13.2 Analytical specificity

The RIDA®GENE Faecalibacterium prausnitzii multiplex real-time PCR is specific for *Faecalibacterium prausnitzii*. No cross-reaction could be detected for the following species (see Tab. 13):

**Tab. 13**: Cross-reactivity testing

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Adenovirus 1, human, strain Adenoid 71 | - | *Campylobacter lari* subsp. *lari* | - | *E. coli* (O157:H7) | - | *Lactobacillus salivaris* | - |
| Adenovirus 7, human, strain Gomen | - | *Campylobacter upsaliensis* | - | *E. coli* (O26:H-) | - | *Proteus vulgaris* | - |
| Adenovirus 40, human, strain Dugan | - | *Candida albicans* | - | *E. coli* (O6) | - | *Pseudomonas aeruginosa* | - |
| Adenovirus 41, human, strain Tak | - | *Citrobacter freundii* | - | *Entamoeba histolytica* | - | Rotavirus | - |
| *Aeromonas hydrophila* | - | *Clostridium bifermentans* | - | *Enterobacter cloacae* | - | *Salmonella enteritidis* | - |
| *Arcobacter butzleri* | - | *Clostridium difficile* | - | *Enterococcus faecalis* | - | *Salmonella typhimurium* | - |
| Astrovirus Type 2 | - | *Clostridium novyi* | - | *Enterococcus faecium* | - | *Serratia liquefaciens* | - |
| Astrovirus Type 8 | - | *Clostridium perfringens* | - | *Giardia intestinalis* Portland 1 | - | *Shigella flexneri* | - |
| *Bacillus cereus* | - | *Clostridium septicum* | - | *Giardia intestinalis* WB Clone C6 | - | *Staphylococcus aureus* | - |
| *Bacteroides fragilis* | - | *Clostridium sordellii* | - | *Giardia lamblia* | - | *Staphylococcus epidermidis* | - |
| *Campylobacter coli* | - | *Clostridium sporogenes* | - | *Klebsiella oxytoca* | - | *Vibrio parahaemolyticus* | - |
| *Campylobacter fetus* subsp. *fetus* | - | *Cryptosporidium muris* | - | *Lactobacillus ruminis* | - | *Yersinia enterocolitica* | - |
| *Campylobacter jejuni* | - | *Cryptosporidium parvum* | - | | | | |

## 14. Version history

| Version number | Chapter and designation |
|---|---|
| 2019-07-01 | General revision<br>3. Test principle<br>4. Reagents provided<br>5. Storage instructions<br>6. Additional necessary reagents and necessary equipment<br>7. Precautions for users<br>8. Collection and storage of samples<br>9. Test procedure<br>10. Quality control<br>11. Result interpretation<br>13. Performance characteristics<br>14. Version history<br>15. Explanation of symbols |

## 15. Explanation of symbols

General symbols

| | |
|---|---|
|  | For in vitro diagnostic use |
|  | Consult instructions for use |
|  | Lot number |
|  | Expiry |
|  | Store at |
|  | Article number |
|  | Number of tests |
|  | Date of manufacture |
|  | Manufacturer |

Testspecific symbols

Not applicable

## 16. Literature


1. Galecka M. *et al. Faecalibacterium prausnitzii* and Crohn's Disease – is There any Connection? Polish Journal of Microbiology 2013, Vol. 62, No 1, 91–95
2. Miquel S. *et al.* Identification of Metabolic Signatures Linked to Anti-Inflammatory Effects of *Faecalibacterium prausnitzii.* mBio 2015, 6(2): e00300-15.
3. Kasper, H. Ernährungsmedizin und Diätetik. Kapitel 3, 162-211. Urban & Fischer Verlag; München/Jena 2000.