

RIDA® GENE *Akkermansia muciniphila*

REF PG0145



1. Intended use

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

2. Summary and explanation of the test

It is becoming more and more apparent that the composition of the gut microbiota affects human health and is associated with different disease conditions including inflammatory bowel diseases (IBDs), metabolic syndrome and obesity.^{3,5} Currently, it is not yet known whether alterations in the microbiota are a cause or an effect of such diseases.

Akkermansia muciniphila (*A. muciniphila*) is a gram-negative, mucin-degrading bacterium and is naturally present in the human microbiota.¹ It represents 3 - 5 % of the bacterial population in a healthy person. *A. muciniphila* is located in special areas of the human mucosa and can be detected in stool samples of infants from the age of less than one month. Afterwards, the quantity of *A. muciniphila* increases with age. In elderly people numbers of *A. muciniphila* are significantly reduced.² *A. muciniphila* can be partially associated with diabetes, adiposity and IBD.^{3,4}

3. Test principle

RIDA[®]GENE Akkermansia muciniphila is a multiplex real-time PCR for the direct, qualitative and quantitative detection *Akkermansia muciniphila* in human stool samples.

After DNA isolation, amplification of the gene fragment (if present) specific for *Akkermansia muciniphila* (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 RIDA[®]GENE Akkermansia muciniphila 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 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 Akkermansia muciniphila 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, LightCycler [®] 480 z
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 and the LightCycler[®] 480 z
- 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 Akkermansia muciniphila 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 DNA extract 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: 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	10 sec, 95 °C
Annealing/Extension	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	15 sec, 95 °C
Annealing/Extension	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 cyclers. A total volume of 5 µl DNA is used resulting in following concentrations:

Standard A: 5×10^2 copies/reaction

Standard B: 5×10^4 copies/reaction

Standard C: 5×10^6 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

<u>Reverse Transcription</u>	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
<u>PCR</u> Denaturation	10 sec, 95 °C
Annealing/Extension	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

<u>Reverse Transcription</u>	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
<u>PCR</u> Denaturation	15 sec, 95 °C
Annealing/Extension	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 cyler. A total volume of 5 µl DNA is used resulting in following concentrations:**

Standard A: 5 x 10² copies/reaction

Standard B: 5 x 10⁴ copies/reaction

Standard C: 5 x 10⁶ copies/reaction

Note: The standard curve can be saved on the real-time PCR cyler for each parameter. Apart from the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cyler, the standard curve is only required to run once per lot number. Using the ABI 7500 (Applied Biosystems) and the CFX96™ (Bio-Rad) cyler 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	<i>Akkermansia muciniphila</i>	530	RIDA® GENE Color Compensation Kit II (PG0002) is required
	ICD	560	
Roche LightCycler® 480II	<i>Akkermansia muciniphila</i>	465/510	RIDA® GENE Color Compensation Kit IV (PG0004) is required
	ICD	533/580	
Roche LightCycler® 480 z	<i>Akkermansia muciniphila</i>	465/510	RIDA® GENE Color Compensation Kit IV (PG0004) is required
	ICD	540/580	
ABI 7500	<i>Akkermansia muciniphila</i>	FAM	Check that passive reference option ROX is none
	ICD	VIC	
Agilent Techn. Mx3005P	<i>Akkermansia muciniphila</i>	FAM	Check that reference dye is none
	ICD	HEX	
Bio-Rad CFX96™	<i>Akkermansia muciniphila</i>	FAM	-
	ICD	VIC	
Qiagen Rotor-Gene Q	<i>Akkermansia muciniphila</i>	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/ μ l. In each PCR run it is used in a total amount of 5×10^3 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	0

**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

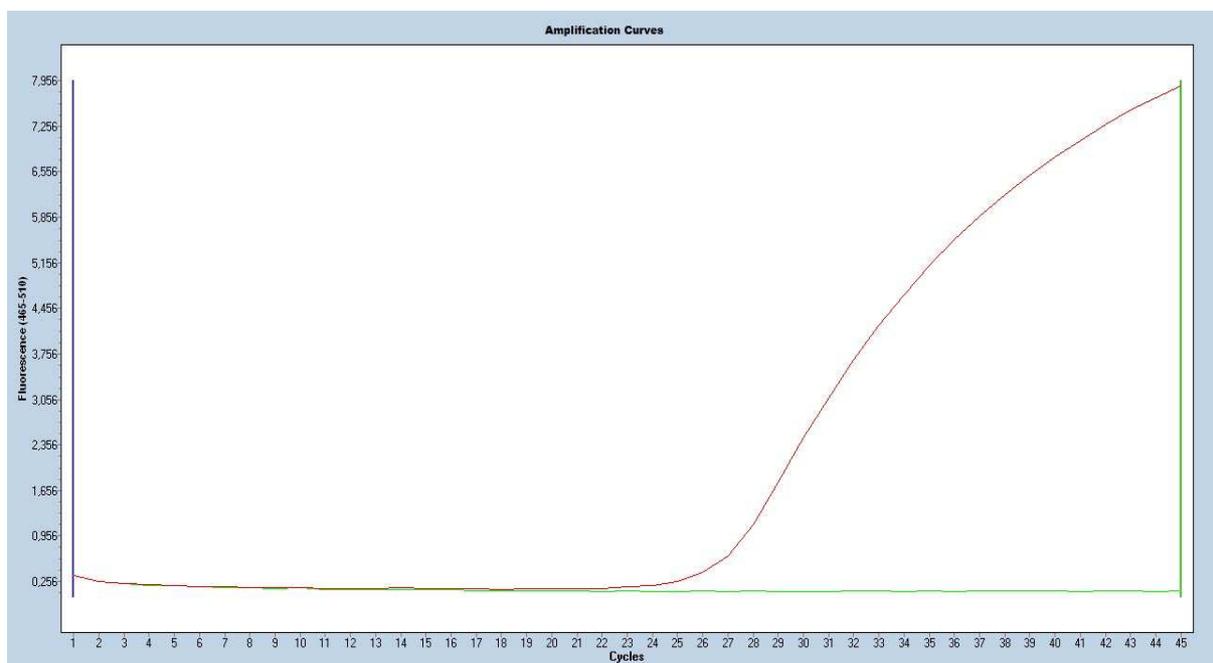


Fig.1: Correct run of the positive control and negative control (*Akkermansia muciniphila*) on the LightCycler® 480II

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	1,896 – 2,102
	Efficiency	1,896 – 2,102
Roche LightCycler® 480II	Slope	-3,1 – -3,6
	Rsq	> 0,98
Agilent Techn. Mx3005P	Y	-3,1 – -3,6
	Eff.	90 % – 110 %
	Slope	-3,1 – -3,6
ABI 7500	R ²	> 0,98
	Eff%	90 % – 110 %
	Efficiency	90 % – 110 %
Bio-Rad CFX96™	Std Curve R ²	> 0,98
	M	3,1 – -3,6
Qiagen Rotor-Gene Q	R ²	> 0,98

11. Result interpretation

The result interpretation is done according to Table 11.

Tab. 11: Sample interpretation

Target genes		
<i>Akkermansia muciniphila</i>	ICD	Result
positive	positive / negative	<i>Akkermansia muciniphila</i> detected
negative*	positive	Target genes not detected*
negative	negative	Invalid

Akkermansia muciniphila is detected, if the sample DNA and the Internal Control DNA show an amplification signal in the detection system.

Akkermansia muciniphila 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.

Akkermansia muciniphila 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 *Akkermansia muciniphila* DNA is unlikely since this bacterial group belongs to the human commensal bacteria. However, there is a low probability that stool samples are by nature negative for *Akkermansia muciniphila*. If a negative result occurs for *Akkermansia muciniphila* 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 *Akkermansia muciniphila* DNA, it is recommended to improve isolation and purification of the sample and repeat amplification.**

11.1 Quantification of samples

To quantify *Akkermansia muciniphila* 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 *Akkermansia muciniphila* 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 *Akkermansia muciniphila* 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 *Akkermansia muciniphila* multiplex real-time PCR in cells/g stool is calculated with following formula:

$$C \text{ [cells/g stool]} = c \text{ [copies/reaction]} \times K$$

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 RIDA® Xtract for sample preparation of a 1:3 diluted sample

Description	Factor
Sample dilution 1:3 before extraction	x 3
200 µl sample for extraction*	x 5
5 µl DNA extract into PCR reaction ((total eluate 60 µl (= 1/12))	x 12
a. Target sequence contained 3x in total Akkermansia-genome	x 1/6 (Akkermansia muciniphila)
Correction factor (K) for Akkermansia muciniphila**	0.6 x 10²

* Result corresponds to 1 g stool

** This value can be saved in the real-time PCR instrument

Tab. 13: 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 3x in total <i>Akkermansia muciniphila</i> genome	x 0.33 (<i>Akkermansia muciniphila</i>)
Correction factor K for <i>Akkermansia muciniphila</i>***	0.66 x 10²

* 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 Akkermansia muciniphila 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).
8. Mucine can show interfering characteristics already in small quantities.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA[®] GENE *Akkermansia muciniphila* multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction for *Akkermansia muciniphila*.

The following figure 2 shows a dilution series of *Akkermansia muciniphila* (10^6 - 10^2 DNA copies per μl) on the LightCycler[®] 480II.

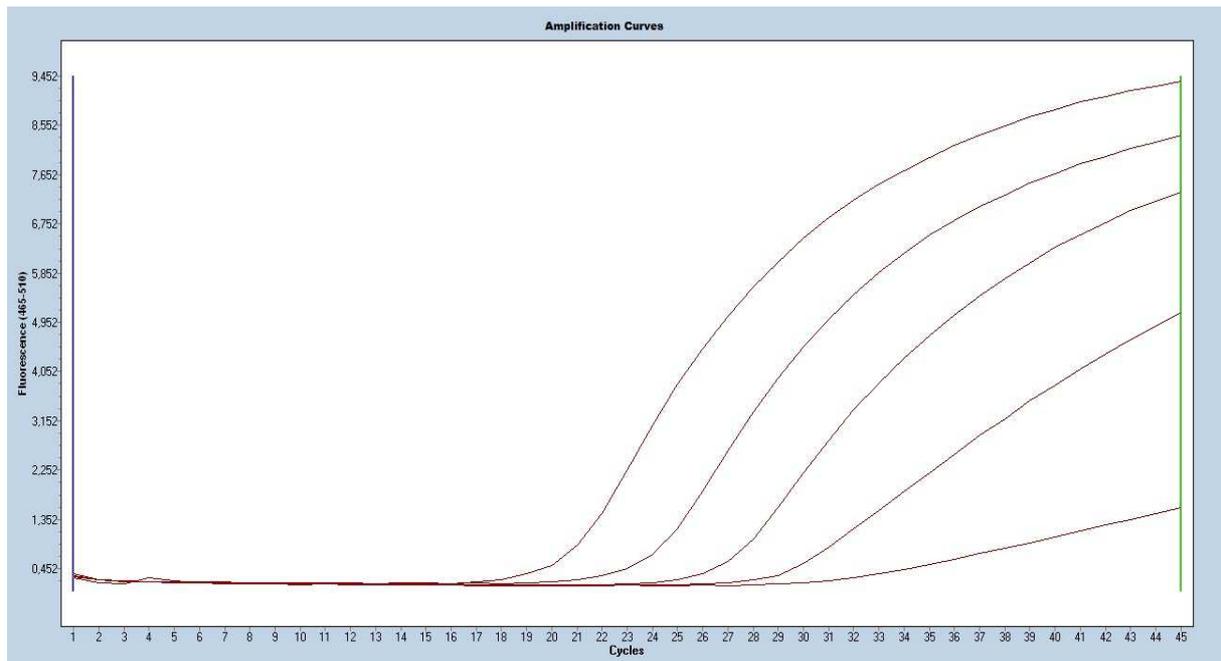


Fig. 2: Dilution series *Akkermansia muciniphila* (10^6 – 10^2 DNA copies per μl) on the LightCycler[®] 480II

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

13.2 Analytical specificity

The RIDA[®] GENE Akkermansia muciniphila multiplex real-time PCR is specific for *Akkermansia muciniphila*. No cross-reaction could be detected for the following species (see Tab. 14):

Tab. 14: Cross-reactivity testing

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

14. Version history

Version number	Chapter and designation
2018-11-12	General revision 4. Reagents provided 5. Storage instructions 6. Additional necessary reagents and necessary equipment 9. Test procedure 10. Quality control 11. Result interpretation 12. Limitations of the method 13. Performance characteristics 14. Version history 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. Derrien M *et al.* Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Sys Evol Microbiology* 2004; 54: 1469-1476.
2. Collado MC *et al.* Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults and the elderly. *App Envir Microbiology* 2007; 73: 7767-7770.
3. Dao MC *et al.* Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* 2015; 0: 1-11.
4. Everard A *et al.* Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *PNAS* 2013; 110: 9066-9071.
5. Cani P *et al.* Next-Generation Beneficial Microbes: The Case of *Akkermansia muciniphila*. *Frontiers in Microbiology* 2017; 8:1765.