RIDA®GENE Dientamoeba fragilis

REF PG1745

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 Dientamoeba fragilis is a multiplex real-time PCR for the direct, qualitative detection of *Dientamoeba fragilis* from human stool samples.[1] The RIDA®GENE Dientamoeba fragilis multiplex real-time PCR is intended for use as an aid in diagnosis of gastrointestinal infection caused by *Dientamoeba fragilis*.

2. Summary and Explanation of the test

Dientamoeba fragilis is one of the most important diarrhea-causing protozoa and is distributed worldwide. Recent studies demonstrated the pathogenic potential and implicated it as a common cause of gastrointestinal disease. Infection with *Dientamoeba fragilis* may be either symptomatic or asymptomatic. Symptoms of dientamoebiasis are abdominal pain and diarrhea. The prevalence of *Dientamoeba fragilis* varies from 0.3 % to 52 % and often exceeds that of *Giardia lamblia*.[2,3] Classically, diagnosis of *Dientamoeba fragilis* is achieved by microscopial examination of faecal samples, which require experienced personal. RIDA®GENE Dientamoeba fragilis multiplex real-time PCR is a new and attractive alternative method for testing stool samples and has proven to be highly sensitive and specific for the detection of *Dientamoeba fragilis*.

3. Test principle

The RIDA®GENE Dientamoeba fragilis is a multiplex real-time PCR for the direct, qualitative detection of *Dientamoeba fragilis* from human stool samples.
After DNA-isolation, amplification of gene fragments (ITS1-18S, if present) specific for *Dientamoeba fragilis* occurs. The amplified target is 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. The RIDA®GENE Dientamoeba fragilis assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and/or to determine possible PCR-inhibition.

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

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 and fully defrost 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 reagents should be stored cold in an appropriate way (2 - 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Dientamoeba fragilis 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 instruments	
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).

- RIDA®GENE Color Compensation Kit II (PG0002) for run the LightCycler® 2.0 (Roche)
- RIDA®GENE Color Compensation Kit IV (PG0004) for run the LightCycler® 480II (Roche)
- 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 (BioScience grade, nuclease-free water).

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

8.1 DNA 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 Dientamoeba fragilis assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient.

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
h as to be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture and **not** 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 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 and the Internal Control DNA 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 Internal Control DNA 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.

Cover tubes or plate. Spin down shortly 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 Annealing/Extension	10 sec, 95 °C 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 and CFX96™

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

Note: Annealing and Extension occur in the same step.

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 Annealing/Extension	10 sec, 95 °C 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, Rotor-Gene Q and CFX96™

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

Note: Annealing and Extension occur in the same step.

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	*Dientamoeba fragilis*	530	**RIDA®GENE Color Compensation Kit II (PG0002) Is required**
	ICD	560	
Roche LightCycler® 480II	*Dientamoeba fragilis*	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) Is required**
	ICD	533/580	
ABI 7500	*Dientamoeba fragilis*	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
Stratagene Mx3005P	*Dientamoeba fragilis*	FAM	**Check that the reference dye is none**
	ICD	HEX	
Qiagen Rotor-Gene Q	*Dientamoeba fragilis*	Green	**-**
	ICD	Yellow	
Bio-Rad CFX96™	*Dientamoeba fragilis*	FAM	**-**
	ICD	VIC	

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 and negative controls have to show correct results (see Fig. 1).

The Positive Control for *Dientamoeba fragilis* 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	0

*[1] *No Ct value is required for the ICR 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

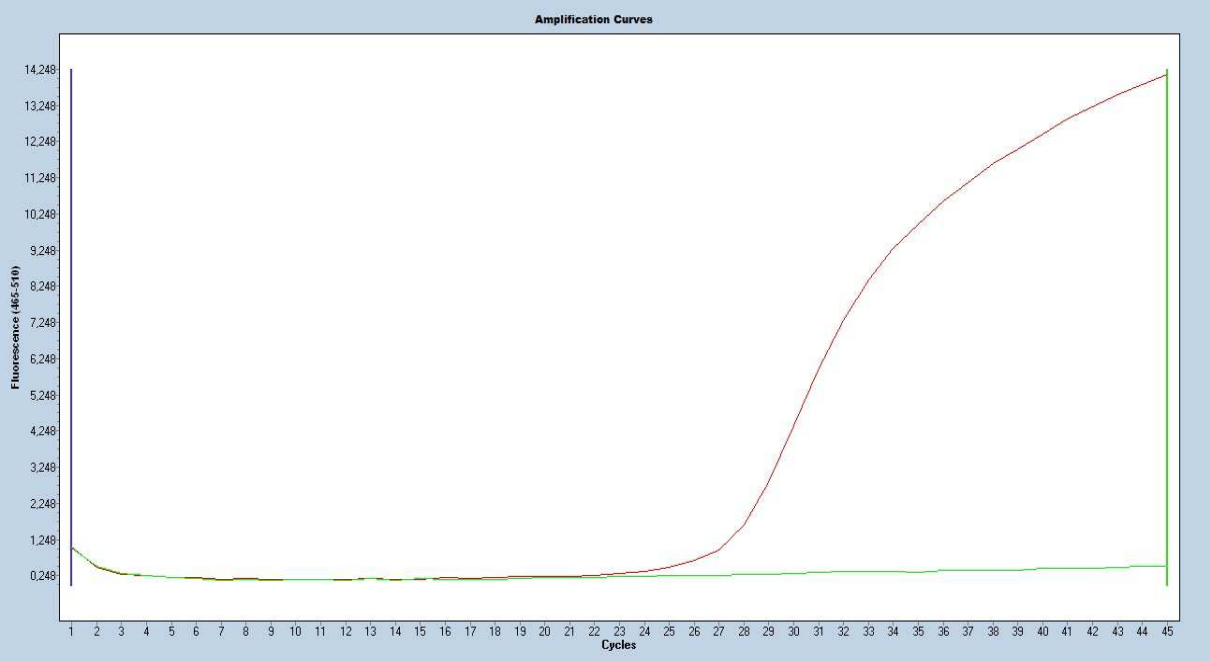

Fig. 1: Correct run of the positive and negative control (*Dientamoeba fragilis*) on theLightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 11.

Tab.11: Sample interpretation

Target genes		
Dientamoeba fragilis	**ICD**	**Result**
positive	**positive/negative**	***D. fragilis* detected**
negative	**positive**	**Target genes not detected**
negative	negative	**Invalid**

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

Dientamoeba fragilis 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.

Dientamoeba fragilis is not detected, if the sample DNA shows no amplification signal, but an amplification signal for the 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.

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 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 Dientamoeba fragilis 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 (ITS1-18S).

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE Dientamoeba fragilis multiplex real-time PCR assay has a detection limit of ≥ 50 DNA copies per reaction for *Dientamoeba fragilis*.

The following figure 2 shows dilution series of *Dientamoeba fragilis* (10^5 - 10^1 DNA copies per µl) on the LightCycler® 480II.

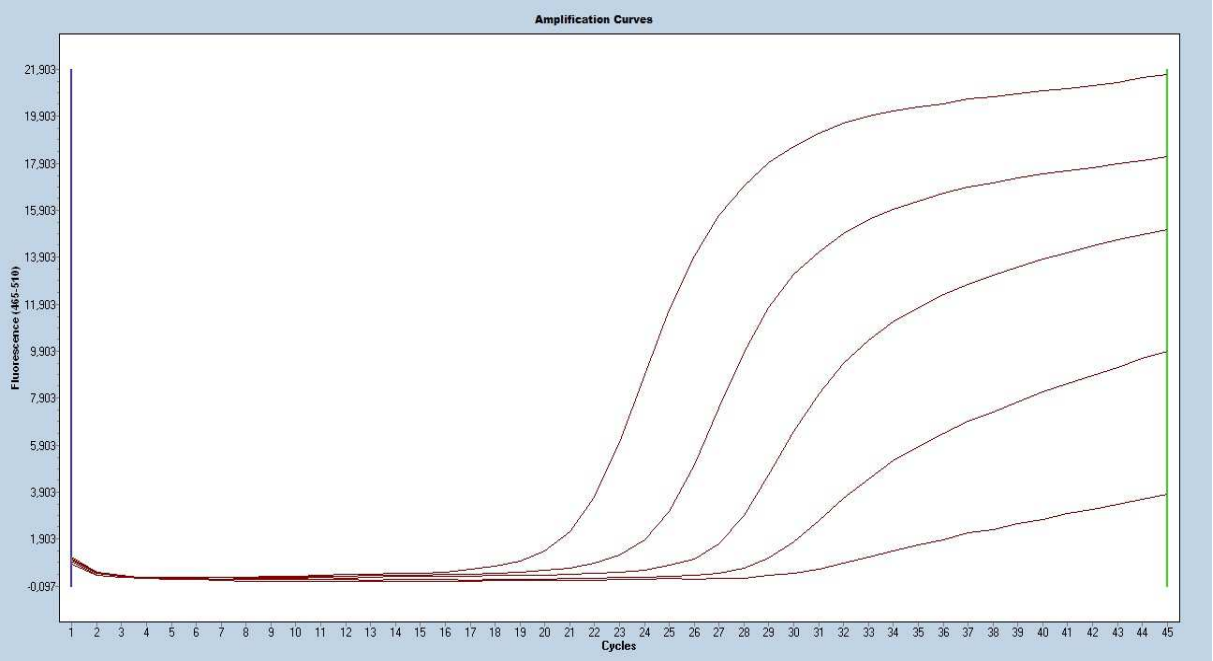

Fig.2: Dilution series *Dientamoeba fragilis* (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

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

13.2 Analytical specificity

The analytical specificity of the RIDA®GENE Dientamoeba fragilis multiplex real-time PCR is specific for *Dientamoeba fragilis* from human stool samples. No cross-reaction could be detected for the following species (see Tab.12).

Tab. 12: Cross-reactivity testing

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

14. Version history

Version number	Chapter and designation
2013-11-12	Release version
2018-07-17	General revision
2018-07-17	4. Reagents provided 5. Storage instructions 6. Additional necessary reagents and necessary equipment 9. Test procedure 10. Quality Control 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. Calderaro A *et al*. Evaluation of a real-time polymerase chain reaction assay for the detection of *Dientamoeba fragilis*. Diagn Microbiol Infect Dis. 2010, 67(3):239-245.
2. Stark D *et al.* A review of the clinical presentation of dientamoebiasis. Am J Trop Med Hyg. 2010, 82(4):614-619.
3. Baratt JLN *et al.* A review of *Dientamoeba fragilis* carriage in humans: several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes. 2011, 2(1):3-12.