RIDA®GENE Lac Intol

REF PY4215

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1. Intended use

For *in-vitro* diagnostic use.
The RIDA®GENE Lac Intol test, which is performed on the LightCycler® 480 II, is a multiplex real-time PCR for the qualitative detection and differentiation of C13910 & G22018 as well as their SNPs (single nucleotide polymorphisms) C13910T & G22018A in the human MCM6 gene from human whole blood EDTA samples from persons with signs and symptoms of lactose intolerance.

The RIDA®GENE Lac Intol test is intended to aid in the diagnosis of patients with symptoms of lactose intolerance in connection with other clinical and laboratory findings.
The test result should not be used as the sole basis for diagnosis.

The product is intended for professional use.

2. Summary and explanation of the test

Lactose, a disaccharide made up of galactose and glucose, is the main energy source of milk in humans and animals.
The β-(1,4) glycosidic bond can be broken down by the enzyme lactase, thereby making the monosaccharides available for further use(1). Breakdown and adsorption take place in the small intestine. The monosaccharides are subsequently transported inside the epithelial cells (enterocytes)(2). The amount of lactase increases during pregnancy and reaches its highest value a few days after birth. Lactase content decreases over a lifetime through regulators at the genetic level(1). Yet 50 % of lactase activity is sufficient to break down lactose effectively(3). If lactose can only be broken down in small quantities or not at all, this leads to an excessive osmotic load and increases the water content in the intestine. Furthermore, lactose gets into the large intestine, where it is fermented by intestinal bacteria, contributing to the production of short-chain fatty acids and gases such as hydrogen, carbon dioxide, and methane(4). This, in turn, can cause clinical symptoms such as bloating, abdominal pain, cramps and/or postprandial fullness, belching, diarrhea and in some cases constipation, nausea, and vomiting(1,4). The gene that codes for lactase is called *LCT* and is located on chromosome 2. The *MCM6* (minichromosome maintenance complex component 6) gene, which is located approx. 14 Kb upstream of the lactase gene, functions as an enhancer. Different single nucleotide polymorphisms (SNPs) including 13910 C/T and 22018 G/A in this gene are associated with lactase persistence. This change leads to new transcription factor binding sites, thus contributing to a lifelong expression of the *LCT* gene(2,4,5). Lactose intolerance is therefore not a disease; it has a global prevalence of 57 % and higher(2,3). This varies from region to region. Europe has an average prevalence of 28 %, varying from 2 % in Scandinavia and 70 % on Sicily(2).

3. Test principle

The RIDA®GENE Lac Intol test is a multiplex real-time PCR for the qualitative detection and differentiation of C13910 & G22018 as well as their possible SNPs (single nucleotide polymorphisms) C13910T & G22018A in the human *MCM6* gene from human whole blood EDTA samples. After DNA isolation, the specific gene fragments are amplified either as a wild type or a mutation.
The amplified target sequences are detected with hydrolysis probes labeled with a quencher at one end and a fluorescent reporter dye (fluorophore) at the other. The probes hybridize to the amplicon in the presence of a target sequence. During extension, Taq-Polymerase separates the reporter from the quencher. The reporter emits a fluorescent signal that is detected by the optical unit of a real-time PCR instrument. The fluorescent signal increases with the quantity of formed amplicons.

4. Reagents provided

The reagents in the kit are sufficient for 100 determinations.

Table 1: Reagents provided

Kit code	Reagent	Amount		Lid color
1	Reaction Mix	2 ×	1050 µL	yellow, ready for use
2	Taq-Polymerase	1 ×	80 µL	red, ready for use
A	Control A	1 ×	200 µL	blue, ready for use
B	Control B	1 ×	200 µL	green, ready for use

5. Storage instructions

- Please follow the handling guidelines in Table 2 and store the kit directly after use according to the information specified.
- All reagents must be stored away from light at -20 °C and, if unopened, can be used until the expiration date printed on the label. After the expiration date, the quality guarantee is no longer valid.
- All reagents should be carefully thawed prior to use (e.g., in a refrigerator at 2 - 8 °C).
- Repeated freezing/thawing of up to 5 times does not have an impact on the test property (if necessary, create aliquots after the first thaw and refreeze reagents immediately).
- Cool all reagents appropriately during PCR preparation (2 - 8 °C).

Table 2: Storage conditions and information

	Storage temperature	Maximum storage time
unopened	-20 °C	Can be used until the printed expiration date
opened	-20 °C	5 freeze-thaw cycles

6. Reagents required but not provided

6.1 Laboratory equipment

The following equipment is needed for using the RIDA®GENE Lac Intol kit:

Equipment
Extraction platform: MagNA Pure 96 instrument (Roche)
Real-time PCR devices: • LightCycler® 480 II (Roche) • ABI 7500 Fast Dx (Applied Biosystems) • CFX96™ Dx (Bio-Rad)
RIDA®GENE Color Compensation Kit IV (PG0004) (R-Biopharm) when using LightCycler® 480 II
Real-time PCR consumables (plates (low profile, white wells, clear frame), reaction vials, slides)
Centrifuge with rotor for plates
Vortexer
Pipettes (0.5 - 20 µL, 20 - 200 µL, 100 - 1000 µL)
Pipette tips with filters
Powder-free disposable gloves

The RIDA®GENE Lac Intol kit can be used in conjunction with compatible workflows. Alternative nucleic acid extraction procedures and real-time PCR devices must be verified/validated by the user. Please contact R-Biopharm AG to review compatibility at pcr@r-biopharm.de.

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.
This test must be carried out only by qualified laboratory personnel. The guidelines for working in medical laboratories must be observed.
Always adhere strictly to the operating manual when carrying out this test.
Do not pipette samples or reagents using your mouth. Avoid contact with broken skin and mucous membranes.
Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test.
Do not smoke, eat, or drink in areas where samples are handled.
Separate rooms, special clothing, and instruments for extraction, PCR preparation, and PCR must be used to prevent cross-contamination and false-positive results.
Avoid contaminating the samples and components of the kit with microbes and nucleases (DNase/RNase).
Clinical samples must be viewed as potentially infectious and must be disposed of appropriately, like all reagents and materials that come into contact with potentially infectious samples.
Do not exchange or mix the components (Reaction Mix, Taq-Polymerase, Control A, Control B) of one lot with the components of another lot.
Do not use the test kit after the expiration date. Users are responsible for the proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

Further details on the Safety Data Sheet (SDS) can be found under the item number at https://clinical.r-biopharm.com/search/.

For users in the European Union: Report all serious adverse events associated with the product to R-Biopharm AG and the appropriate national authorities.

8. Collection and storage of samples

8.1 Sample storage

This test was developed for the examination of human whole blood EDTA samples. The samples must be stored at room temperature for up to 24 hours and 2 - 8 °C for up to 72 hours until the DNA is extracted[6]. Microbial contamination of the samples is to be avoided. The use of lipemic, hemolytic, icteric or opaque serums inactivated by heat may lead to distorted results.

8.2 Sample preparation

8.2.1 DNA isolation from whole blood EDTA

A commercially available DNA isolation kit or DNA extraction system (e.g., MagNA Pure 96 instrument (Roche)) is recommended for the isolation of DNA from whole blood EDTA. The manufacturer's instructions must be observed.
When using the MagNA Pure 96 instrument (Roche), extract 200 µL of whole blood EDTA DNA using the DNA/Viral NA SV kit and the Pathogen Universal 200 protocol. Elute the DNA with 50 µL of elution buffer.

9. Test procedure

9.1 Preparation of the Master Mix

The total number of the reactions needed for PCR (samples and control reactions) must be calculated. Control A and Control B must be included for each test run.

Adding an additional 10 % volume to the Master Mix is recommended in order to compensate for any pipetting loss (see Table 3). Prior to use, thaw the Reaction Mix, the Taq-Polymerase, Control A and Control B, vortex (except Taq-Polymerase), and briefly centrifuge. Reagents must always be cooled appropriately during the work steps (2 - 8 °C).

Table 3: Example for the calculation and production of the Master Mix for 10 reactions

Kit code	Components of the Master Mix	Quantity per reaction	10 reactions (plus 10 %)
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 Master Mix and then centrifuge for short time.

9.2 Preparation of the PCR mix

Pipette 20 µL of the Master Mix into each reaction vial (plates).

Samples: Add 5 µL eluate to each pre-pipetted Master Mix.

Controls: Add 5 µL of Control A to each pre-pipetted Master Mix.

Add 5 µL of Control B to each pre-pipetted Master Mix.

Seal the plates, briefly centrifuge at slow speed, and transfer to the real-time PCR instrument. Start PCR according to PCR instrument setup (see Table 4, Tab. 5).

9.3 Device settings

9.3.1 Universal real-time PCR profile

To harmonize the RIDA®GENE assays, the RIDA®GENE Lac Intol kit was verified in the universal profile. This makes it possible to combine DNA and RNA assays with each other. Reverse transcription therefore comes first in the universal profile.

Table 4: Universal real-time PCR profile for LightCycler® 480 II

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 take place in the same step.**

Table 5: Universal real-time RT-PCR profile for ABI 7500 Fast Dx and CFX96™ Dx

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 take place in the same step.**

9.4 Detection channel setting

Table 6: Selection of appropriate detection channels

Real-time PCR device	Detection	Detection channel	Note
Roche LightCycler® 480 II	C13910 (WT)	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required.**
	C13910T (Mut)	533/580	
	G22018 (WT)	533/610	
	G22018A (Mut)	618/660	
Applied Biosystems ABI 7500 Fast Dx	C13910 (WT)	FAM	**Set the ROX passive reference dye to none.**
	C13910T (Mut)	VIC	
	G22018 (WT)	ROX	
	G22018A (Mut)	Cy5	
Bio-Rad CFX96™ Dx	C13910 (WT)	FAM	-
	C13910T (Mut)	VIC	
	G22018 (WT)	ROX	
	G22018A (Mut)	Cy5	

10. Quality control - indication of instability or expiration of reagents

Samples are evaluated using the analysis software of the real-time PCR instrument according to the manufacturer's instructions. Control A and Control B must indicate the correct results (see Table 7).

Control A and Control B are each present in a concentration of 10^3 copies/µL. It is used in a total quantity of 5 x 10^3 copies in every PCR run.

Table 7: A valid PCR run must meet the following conditions:

Sample	FAM	ROX	VIC	Cy5	Target gene Ct
Control A	+	+	-	-	See Certificate of Analysis
Control B	-	-	+	+	See Certificate of Analysis

* + = positive
  - = negative

If both controls, Control A and Control B, are not in line with the specifications, the whole PCR run must be repeated. If the specified values are not met, check the following items before repeating the test:

- Expiration date of the reagents used
- Functionality of the equipment being used
- Correct test procedure

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Result interpretation

The sample results are interpreted according to Table 8.

Table 8: Result interpretation*

Detection of				
C13910 (WT)	**C13910T** (Mut)	**G22018** (WT)	**G22018A** (Mut)	**Result**
+	-	+	-	**Homozygous wild type 13910** **Homozygous wild type 22018**
+	-	+	+	**Homozygous wild type 13910** **Heterozygous 22018**
+	+	-	+	**Heterozygous 13910** **Homozygous mutation 22018**
+	+	+	-	**Heterozygous 13910** **Homozygous wild type 22018**
+	+	+	+	**Heterozygous 13910** **Heterozygous 22018**
-	+	+	-	**Homozygous mutation 13910** **Homozygous wild type 22018**
+	-	-	+	**Homozygous wild type 13910** **Homozygous mutation 22018**
-	+	-	+	**Homozygous mutation 13910** **Homozygous mutation 22018**
-	+	+	+	**Homozygous mutation 13910** **Heterozygous 22018**
-	-	+ / -	+ / -	**invalid**
+ / -	+ / -	-	-	**invalid**
-	-	-	-	**invalid**

* + = positive
  - = negative

Note: When the LightCycler® 480 II (Roche), the CFX96™ Dx (Biorad) and the ABI 7500 Fast Dx (Applied Biosystems) are used, the fluorescence level of a truly positive signal in the VIC channel must be at least 20 % of the fluorescence signal of Control B. For a more definitive evaluation, it is recommended to set the threshold to this limit (20 % Control B).

The PCR run cannot be evaluated if the controls display no amplification in the detection system. The whole PCR run must be repeated.

The PCR run cannot be evaluated if the sample displays no amplification in the detection system. In this case, either the DNA was not added or an unsuitable

template DNA (quality, PCR inhibitor) was used. The extracted sample should be re-amplified or the isolation and cleaning of the sample should be improved.

12. Limitations of the method

1. The RIDA®GENE Lac Intol test detects C13910 & G22018 and/or their possible SNPs (single nucleotide polymorphisms) C13910T & G22018A in the human *MCM6* gene from human whole blood EDTA samples. A connection between the level of the determined Ct value and the occurrence of severe clinical symptoms cannot be derived from this. The results obtained must always be interpreted in combination with the complete clinical symptoms.
2. The diagnosis should not be based on the result of the molecular biological analysis alone, but should always take the patient's medical history and symptoms into account.
3. This test is valid only for human whole blood EDTA samples.
4. Improper specimen extraction, transport, storage, and handling can produce false-negative results.
5. The presence of PCR inhibitors can lead to false-negative or invalid results.
6. Individual base exchanges are detected for the SNP technology used. This can adversely affect the endpoint fluorescence level.
7. This assay should be performed in compliance with the regulation on good laboratory practice Users must precisely follow the manufacturer's instructions when performing the test.
8. The German Genetic Diagnostics Act (GenDG) requires a thorough explanation and written consent of the patients for all genetic analyses in accordance with the GenDG.

13. Performance characteristics

13.1. Analytical performance characteristics

13.1.1 Analytical specificity

Interfering substances

The presence of PCR inhibitors and interfering substances can lead to invalid results. Therefore the effects of different substances that could be present in the respective samples because of their widespread use should be tested.
Substances that could potentially significantly influence the test results were examined at high concentrations (simulation of the worst case) in an interference screen.
No interferences were determined for the substances listed in Table 9.

Table 9: Potentially interfering substances

Potentially interfering substance	Concentration
Medunasal Heparin 500 I.U. Ampules (heparin)	15 U/mL
Cholesterol	3 mg/mL
Bilirubin	0.1 mg/mL
Hemoglobin	0.2 mg/mL
K_2EDTA	1.8 mg/mL

13.1.2 Precision

The precision of the Lac Intol kit was determined for the following assessment levels.

Intra-assay precision: Determination of 7 control samples using 20 replicates each on LightCycler® 480 II under identical conditions.

Inter-assay precision: Determination of 7 control samples in 20 runs in duplicate on 10 work days (2 runs per day) performed by two different technicians under reproducible conditions.

Testing for intra- and inter-assay precision was carried out using three different lots.

The precision data were determined with seven control samples as well as the control A and control B associated with the assay.

The maximum obtained coefficient of variation of the measurements with the RIDA®GENE Lac Intol kit on LightCycler® 480 II was 8.72 %.

Table 12: Results of the precision of the Lac Intol kit for the FAM channel.

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1-3
1	Ct	23.3	23.4	22.9	23.6	24.2	24.2	24.0
	CV (%)	0.84 %	1.10 %	2.52 %	3.34 %	2.46 %	2.27 %	2.99 %
2	Ct	23.4	23.6	23.2	23.8	24.1	24.3	24.1
	CV (%)	1.46 %	0.95 %	1.80 %	2.22 %	2.37 %	2.20 %	2.44 %
3	Ct	23.6	23.8	23.6	23.9	24.2	24.6	24.2
	CV (%)	0.67 %	1.02 %	1.18 %	2.80 %	2.22 %	2.34 %	2.83 %
4	Ct	24.4	24.6	24.5	24.3	24.5	25.0	24.6
	CV (%)	1.41 %	1.01 %	1.14 %	2.91 %	2.46 %	2.63 %	2.96 %
5	Ct	24.1	24.2	23.9	24.0	24.3	24.6	24.3
	CV (%)	0.88 %	1.13 %	0.96 %	2.78 %	2.42 %	2.01 %	2.59 %
6	Ct	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
7	Ct	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 13: Results of the precision of the Lac Intol kit for the VIC channel.

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1-3
1	Ct	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Ct	28.0	27.0	27.5	28.4	28.9	28.1	28.5
	CV (%)	1.81 %	2.13 %	3.62 %	2.71 %	2.70 %	4.38 %	3.58 %
3	Ct	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
4	Ct	31.2	29.4	28.9	28.5	29.1	28.3	28.7
	CV (%)	5.35 %	8.72 %	1.75 %	2.42 %	2.03 %	3.86 %	3.15 %
5	Ct	28.7	28.0	27.3	27.2	27.3	27.0	27.2
	CV (%)	2.41 %	6.02 %	3.44 %	1.75 %	1.73 %	2.84 %	2.21 %
6	Ct	27.5	26.5	26.8	26.9	26.9	26.8	26.9
	CV (%)	1.40 %	1.33 %	0.97 %	1.30 %	1.46 %	1.34 %	1.37 %
7	Ct	25.9	25.8	25.6	26.5	26.7	26.2	26.5
	CV (%)	0.87 %	1.36 %	0.88 %	1.63 %	1.04 %	1.75 %	1.75 %

Table 14: Results of the precision of the Lac Intol kit for the ROX channel.

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1-3
1	Ct	23.4	23.2	23.3	24.0	24.4	24.5	24.3
	CV (%)	0.95 %	1.08 %	1.89 %	2.47 %	2.16 %	2.07 %	2.46 %
2	Ct	23.7	23.5	24.0	24.4	24.5	24.8	24.6
	CV (%)	1.00 %	0.81 %	1.56 %	1.98 %	2.07 %	1.69 %	2.06 %
3	Ct	23.6	23.4	23.9	24.2	24.4	24.9	24.5
	CV (%)	0.64 %	1.35 %	1.46 %	2.43 %	2.25 %	2.23 %	2.61 %
4	Ct	24.7	25.1	24.7	24.8	25.0	25.3	25.1
	CV (%)	1.22 %	1.24 %	1.28 %	2.52 %	2.34 %	2.33 %	2.53 %
5	Ct	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6	Ct	24.2	24.4	24.0	24.4	24.4	24.6	24.4
	CV (%)	0.94 %	1.32 %	1.10 %	2.71 %	2.40 %	2.60 %	2.57 %
7	Ct	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 15: Results of the precision of the Lac Intol kit for the Cy5 channel.

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1-3
1	Ct	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Ct	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	Ct	25.5	26.0	28.3	25.7	26.1	27.0	26.3
	CV (%)	1.06 %	1.54 %	4.10 %	2.74 %	3.19 %	3.69 %	4.06 %
4	Ct	25.9	26.2	26.2	26.2	26.4	27.4	26.7
	CV (%)	1.62 %	2.90 %	1.54 %	3.48 %	2.86 %	3.19 %	3.95 %
5	Ct	25.3	25.6	25.7	25.7	25.8	26.5	26.0
	CV (%)	0.95 %	1.84 %	0.61 %	2.17 %	2.11 %	1.75 %	2.55 %
6	Ct	25.5	25.8	25.7	26.0	26.0	26.4	26.1
	CV (%)	1.29 %	1.03 %	0.99 %	3.33 %	2.59 %	3.05 %	3.07 %
7	Ct	25.4	25.5	25.8	25.4	25.5	26.1	25.7
	CV (%)	0.90 %	1.16 %	0.86 %	2.28 %	1.94 %	2.28 %	2.58 %

14. Version history

Version number	Section and designation
2022-05-11	Release version

15. Explanation of symbols

General symbols

IVD	For *in vitro* diagnostic use
	Observe operating manual
LOT	Batch number
	Use before
	Storage temperature
REF	Item number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Reaction Mix	Reaction Mix
Taq-Polymerase	Taq Polymerase
Control A	Control A
Control B	Control B

16. References

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