Genvinset®

Lactose Intolerance

INSTRUCTIONS FOR USE

Kit for detection of C13910T and G22018A polymorphisms in the MCM6 gene

For In Vitro Diagnostic use

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Camino del Pilón 86, Casa 7, Local
50011 – Zaragoza (Spain)

www.bdrdiagnostics.com

CE

Product codes:

GVS-LAC-24

GVS-LAC-48

UDI-DI:

8437016942529

8437016942536

Store

from -30°C to -18°C

Genvinset®

Lactose Intolerance

Index

1- Information for safety

Please, read completely these instructions for use and follow them during the use of the current IVD kit.

The IVD kit shall be used by experts with strong experience in DNA analysis and interpretation of genetic results.

Consult the manufacturer if there are doubts involving the description of the assay method. Contact by phone +34 976 094 603 or email address *customersupport@bdrdiagnostics.com*.

The IVD kit has a limited shelf life. Make sure that the shelf life is not expired before using the kit. Reagents from a kit beyond its expiry date might be degraded, which could impair results. Discard the expired reagents following applicable regulations.

The contamination of the sample or the reagent can render incorrect results. Be careful during the DNA extraction and sample and reagents manipulation.

This kit can be damaged during transport or storage. Do not use the kit in case of suspected damage during transport. Follow carefully the storage conditions described in the label and the IFU.

Ensure that the waste is managed according to local regulations. Incorrect waste management can result in environmental contamination.

The toxicological properties of the kit have not been studied in-depth, so it is recommended to avoid contact with skin and mucous membranes. Do not ingest. Manual Safety Data Sheets (MSDS) are available for the customers by request.

Ensure that this kit is adequate for the requested analysis by the clinician.

2- Intended Use

Genvinset® Lactose Intolerance is a semi-automated *in vitro* diagnostic kit for the qualitative detection of the polymorphisms -13910 C/T (NCBI dbSNP rs4988235; NM_005915.6: c.1917+326C>T) and -22018 G/A (NCBI dbSNP rs182549; NM_005915.5: c.1362+117G>A) of the *MCM6* gene (OMIM: 601806) associated with lactase persistence, in genomic DNA extracted from whole blood, by Real Time PCR using specific TaqMan® probes technology.

Patients who can benefit from this determination are those referred by a specialist. The results of this test should not be the only ones on which the therapeutic decision is based and should be used as an aid in the diagnosis together with results of other markers of the disease.

The intended user of the kit is technical personnel trained to carry out the protocol and the interpretation of results described in this document.

3- Summary and explanation

Lactose is a disaccharide found in mammalian milk, that is hydrolysed in the intestine by the lactase enzyme (also known as lactase-phlorizin hydrolase or LPH), into two absorbable monosaccharides, glucose and galactose. In most children, lactase activity is at its peak during the perinatal period and is essential during breastfeeding. However, after a few months its activity gradually decreases to almost undetectable levels due to a natural downregulation of lactase expression (primary hypolactasia)[1].

However, some humans maintain lactase activity into adulthood, and thus two groups of people can be found: a "lactase non-persistence" group with low lactase activity, and a "lactase persistent" group who sustain the ability to digest milk[2,3]. The frequency of lactase persistence varies greatly depending on the region: it is high in North-European populations (>90% in Sweden and Denmark), whereas it progressively decreases towards the south of Europe and the Middle East (around 50% in France, Spain and some Arabic populations), and is very low in Asian and African populations, although it is common in pastoralist populations (around 1% in China, 5-20% in East Africa)[4].

Low lactase activity during adulthood leads to maldigestion of lactose, producing symptoms such as abdominal pain and distension, bloating, flatulence, and diarrhoea[1]. However, many people with lactase non-persistence can tolerate small amounts of lactose without symptoms, and tolerance can be acquired by adaptation of the intestinal flora with regular lactose intake (although lactase expression does not change)[5]. Therefore, not all patients with lactase deficiency develop lactose intolerance.

The lactase gene (*LCT*) is located on the long arm of chromosome 2. Two single nucleotide polymorphisms upstream of *LCT* have been associated with lactase persistence:

- **LCT-13910 C/T** is located in intron 13 of the minichromosome maintenance type 6 gene (*MCM6*), It is inherited in an autosomal dominant way, with one allele being sufficient to confer the lactase persistence phenotype. It is generally accepted that the genotyping of -13910 C/T polymorphism correlates accurately with lactase persistence in European populations (where it is positively correlated in ~86%-98% of cases) and should therefore be used in the diagnosis of primary hypolactasia[6,7]. However, this polymorphism is not a good indicator of lactase persistence in non-Europeans, since pastoralist populations of Africa and Asia, although they show a high prevalence of lactase persistence, present a low frequency of the -13910T allele[8].
- **LCT-22018 G/A** is located in intron 9 of *MCM6*. This polymorphism is a better indicator of lactose persistence In Northern China and Japanese Brazilian populations[9,10], and is found in association with -13910 C/T in most cases, with some exceptions[2,9].

The polymorphisms associated with lactase persistence are dominant and lactase activity remains with a single lactase-persistent allele[11]. However, there have been reported some minority cases of heterozygous patients for the -22018 G/A polymorphism that exhibit lactase non-persistence[12-14].

<table>
<tr><th>Polymorphism</th><th colspan="3">Genotype and phenotype</th></tr>
<tr><td rowspan="2">-13910 C/T</td><td colspan="2">Lactase persistence</td><td>Lactase non-persistence (Lactose intolerant)</td></tr>
<tr><td>T/T</td><td>C/T</td><td>C/C</td></tr>
<tr><td rowspan="2">-22018 G/A</td><td>Lactase persistence</td><td>Mainly associated with lactase persistence[12]</td><td>Lactase non-persistence (Lactose intolerant)</td></tr>
<tr><td>A/A</td><td>G/A</td><td>G/G</td></tr>
</table>

4- Procedure principles

The test is based on real-time PCR technology and TaqMan® probes. Each sample is analysed in two reactions carried out in two different wells (Reaction C13910T and Reaction G22018A). The following polymorphisms are analysed in each reaction:

Reaction	Polymorphism	FAM channel	HEX channel
C13910T	-13910 C/T (rs4988235)	Allele T	Allele C
G22018A	-22018 G/A (rs182549)	Allele A	Allele G

In Reaction C13910T, each sample is analysed using:

- A pair of primers for the amplification of a fragment of the *MCM6* gene where the -13910 C/T polymorphism is located.
- A hydrolysis probe specific for the wild-type allele (C at rs4988235) labelled at the 5' end with HEX fluorophore and a hydrolysis probe specific for the mutant allele (T at rs4988235) labelled at the 5` end with FAM fluorophore.

In Reaction G22018A, each sample is analysed using:

- A pair of primers for the amplification of a fragment of the *MCM6* gene where the -22018 G/A polymorphism is located.
- A hydrolysis probe specific for the wild-type allele (G at rs182549) labelled at the 5' end with HEX fluorophore and a hydrolysis probe specific for the mutant allele (A at rs182549) labelled at the 5` end with FAM fluorophore.

All probes are labelled at the 3' end with a quencher that suppresses the fluorescence of the fluorophores when the probe is intact.

As the PCR reaction proceeds, the 5'→3'exonuclease activity of Taq polymerase cleaves the probes attached to their complementary sequence, separating fluorophore from quencher, and producing a fluorescent signal (in real time), which is proportional to the amount of PCR product generated and monitored in a real-time PCR instrument. Thus:

- In the case of homozygous wild-type (wt) samples for the analysed changes (-13910 C/C or -22018 G/G), the HEX-labelled wt allele-specific probe binds to its complementary sequence of the amplified gene, and the following is observed:
    - fluorescent signal in the HEX channel (λmax 556 nm), and
    - no signal or weak signal in the FAM channel (λmax 518 nm).
- In the case of homozygous mutant (mut) samples for the analysed changes (-13910 T/T or -22018 A/A), the FAM-labelled mut allele-specific probe binds to its amplified complementary sequence. In this case, the following is detected:
    - fluorescent signal in the FAM channel and
    - no signal or weak signal in the HEX channel.
- In the case of heterozygous samples for the analysed change (-13910 C/T or -22018 G/A), both probes shall bind to the amplified DNA sequences generating:
    - FAM channel signal and
    - signal in the HEX channel.

5- Kit contents

➔ GVS-LAC-24 (24 tests)

- GVS-CT139-PM: 1 vial x 96 µL Primer Mix C13910T (PM) - Blue cap
- GVS-CT139-MM: 1 vial x 120 µL Master Mix C13910T (MM) - Red cap
- GVS-GA220-PM: 1 vial x 96 µL Primer Mix G22018A (PM) - Blue cap
- GVS-GA220-MM: 1 vial x 120 µL Master Mix G22018A (MM) - Red cap

- GVS-LAC-C1: 1 vial x 15 µL Control WT (C1) - Green cap
- GVS-LAC-C2: 1 vial x 15 µL Control MUT (C2) - Green cap with an orange sticker
- GVS-RB: 1 vial x 100 µL Reaction Blank (RB) - Natural cap

➔ **GVS-LAC–48 (48 tests)**

- GVS-CT139-PM: 2 vials x 96 µL Primer Mix C13910T (PM) - Blue cap
- GVS-CT139-MM: 2 vials x 120 µL Master Mix C13910T (MM) - Red cap
- GVS-GA220-PM: 2 vials x 96 µL Primer Mix G22018A (PM) - Blue cap
- GVS-GA220-MM: 2 vials x 120 µL Master Mix G22018A (MM) - Red cap
- GVS-LAC-C1: 1 vial x 15 µL Control WT (C1) - Green cap
- GVS-LAC-C2: 1 vial x 15 µL Control MUT (C2) - Green cap with an orange sticker
- GVS-RB: 1 vial x 100 µL Reaction Blank (RB) - Natural cap

6- Kit storage

All the components of the kit must be stored between -30°C and -18°C upon receipt. Under these conditions, they are stable up to their expiry date.

Do not perform more than 3 freeze/thaw cycles on the kit vials as this may reduce the sensitivity of the assay and affect the results. If assays are to be performed with few samples, it is recommended to use aliquots of the reagents to reduce freeze/thaw cycles.

Due to the photosensitive nature of the Primer Mix, avoid continuous exposure to light.

7- Materials required but not supplied

General

- Disposable Gloves
- Lab coat

Consumables

- Filter tips (P200, P20 and P10)
- 1.5 mL autoclaved tubes
- Compatible consumables for the real-time PCR instrument

Equipment

- Vortex mixer
- Centrifuge
- Pipettes (P200, P100 & P10)
- Real-time PCR instrument with FAM and HEX/VIC detection channels. The following devices have been validated:
    - StepOne™, 7500 Real-Time PCR System, and QuantStudio™ 5 Dx Real-Time PCR Systems, Applied Biosystems™
    - LightCycler® 96 System, Roche
    - CFX96, BioRad
    - DT Lite Real-Time PCR System, DNA-Technology

8- Sample collection and preparation

Samples must be collected in line with the instructions for use of the collection device (not included with the kit) and under national and international guidelines.

The present test should only be performed with genomic DNA extracted from whole blood samples preserved with anticoagulant agents such as EDTA or citrate. Heparin can interfere with the PCR process and should be avoided.

The technique is compatible with several DNA extraction methods. Before delivering results with a diagnostic purpose, a validation assay with such extraction method should be done.

CAUTION!

All biological and blood samples should be treated as potentially infectious. When manipulating them, take the corresponding basic and universal precautions.

9- Usage procedures

➔ PCR setup

CAUTION!

- Define pre- and post-PCR work areas that should be kept separated to reduce the risk of contamination. Prepare the PCR in the pre-PCR area. Use lab coat and disposable gloves.
- Work on ice or over a cool block. Minimize the time between preparation and start of the assay.
- For each assay, it is recommended to test in both Reactions (C13910T and G22108A) the contamination control (Reaction Blank) and both the Control WT (C1) and Control MUT (C2) included in the kit.

1. Thaw all the kit components before starting the assay. Vortex vigorously the Primer Mix vials and mix carefully the Master Mix vials. Centrifuge briefly to collect the volume at the bottom of the tubes.
2. For each reaction (C13910T and G22018A), prepare the reaction mix for n+1 samples, using the quantities indicated in the following table:

	C13910T reaction Vol. per sample (µL)	G22018A reaction Vol. per sample (µL)
Primer Mix	4	4
Master Mix	5	5

   Gently mix and centrifuge to ensure that all volume settles to the bottom of the tubes.
3. Pipette 9 µL of each mix into the PCR plate/tubes.
4. Add into each well 1 µL of DNA, Reaction Blank, Control WT or Control MUT. As there are two reactions, two wells per sample are used.
5. Seal the plate/tubes using the appropriate sealer and centrifuge briefly to remove any bubbles. Ensure that all the volume settles to the bottom of the well.

6. Place the plate/tubes in the thermal cycler and set up the thermal cycler amplification program as described in the following section.

➔ Thermal cycler configuration

1. Set up the following readout channels:
    - FAM channel for detection of the mut probe.
    - HEX/VIC channel for detection of the wt probe.
2. Set up the following amplification program and start the run:

	Cycles	Temperature (°C)	Time (mm:ss)	Analysis
Denaturation	1	95	05:00	X
Cycles	40	95	00:15	X
		60	01:00	Single
Cooling	1	15	∞	X

➔ Disposal

Waste products shall be managed according to local regulations.

10- Results

➔ Results visualization

The analysis of the results is performed with the specific software of the real-time PCR instrument being used, and according to the manufacturer's instructions for use.

Once the run is finished, select the linear scale to visualize the amplification curves. It is recommended to check the correct behaviour of the obtained amplification curves:

- An amplification signal is considered positive if a quick and regular increase of fluorescence values (exponential) is observed (sigmoidal amplification) with Ct<35.
- A weak fluorescent, background signal or exponential signal with Ct>35 should not be considered a positive amplification. This assay allows the detection of alleles that differ by only one nucleotide and, therefore, in samples homozygous for one of the alleles, weak non-specific signals from the fluorophore used for the detection of the other allele can be observed. The appearance of these signals does not invalidate the assay.

A sample is considered positive if it produces an exponential amplification with Ct<35. A sample is considered negative if it produces a non-exponential amplification with low intensity or an exponential amplification with a Ct value >35.

CAUTION!

To determine the Ct value in each channel, adjust the threshold line as follows:

Select linear scale view and choose the region where the fluorescence signal is stable before exponential amplification. Place the threshold line above this background signal, so that it crosses close to the inflection point of the amplification curve. This line should slightly exceed the value of highest fluorescence obtained with negative samples for the allele detected in this channel.

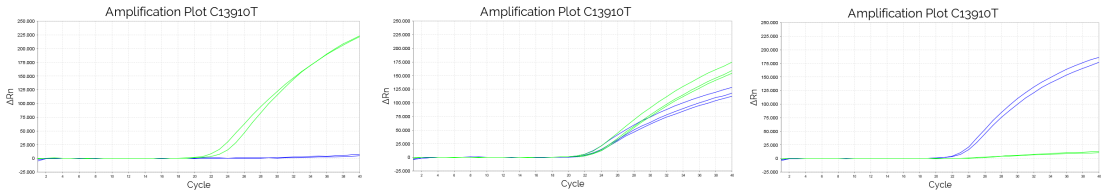
➔ Interpretation of results

The results obtained with this kit can be interpreted by visualization of the amplification curves in the FAM and HEX channels or by scatter plot of the end-point intensity values of the FAM and HEX channels.

Amplification curve

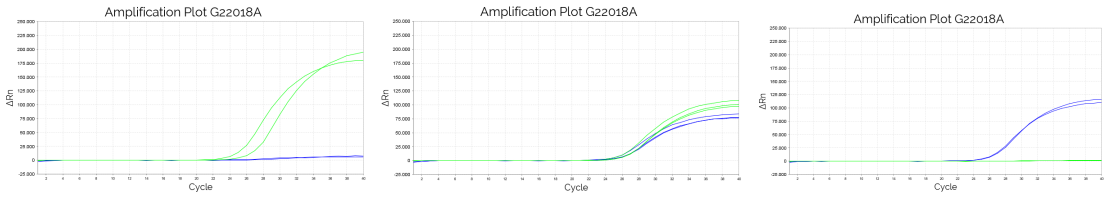
Select "linear scale" and observe the absence/presence of sigmoid amplification curves in each channel.

Reaction C13910T



Amplification curves for homozygous wt (-13910 C/C) (left), heterozygous (-13910 C/T) (middle) and homozygous mut (-13910 T/T) (right) samples. FAM channel (blue line), HEX channel (green line)

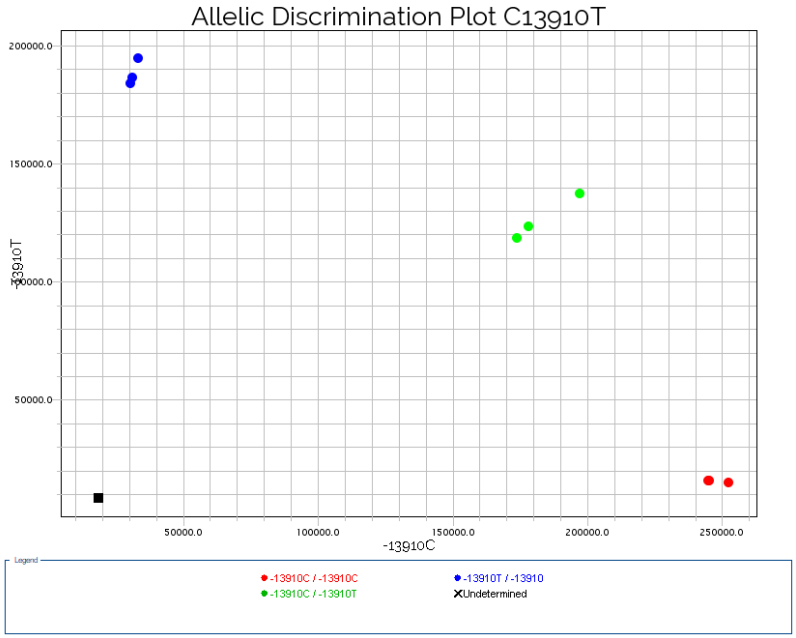
Reaction G22018A



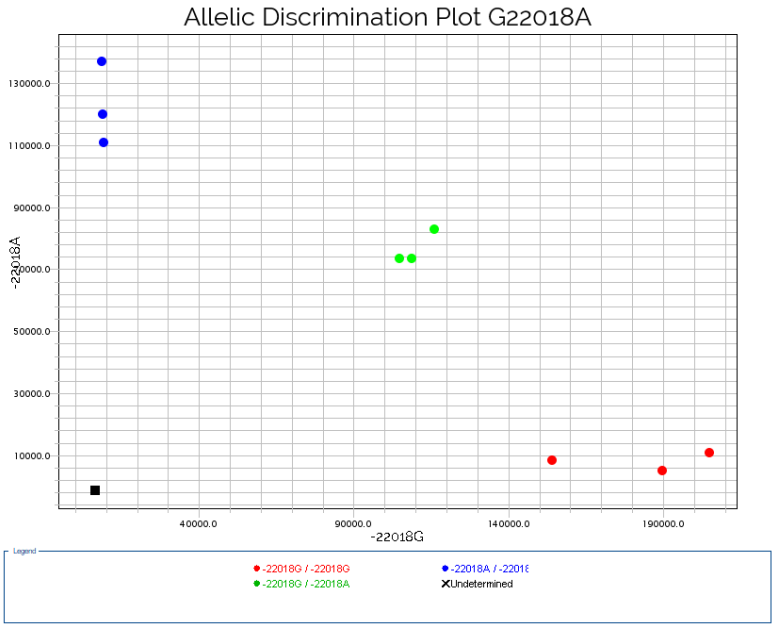
Amplification curves for homozygous wt (-22018 G/G) (left), heterozygous (-22018 G/A) (middle) and homozygous mut (-22018 A/A) (right) samples. FAM channel (blue line), HEX channel (green line)

Scatter plot

Many real-time PCR software programs allow to automatically plot the fluorescence intensity data of one channel versus the other (allelic discrimination/genotyping). In this type of representation, data points located close to the X and Y axes correspond to homozygous genotypes for the allele detected with the fluorophore represented on the corresponding axis. Points located approximately in the middle of the axis correspond to heterozygous genotypes and the negative control (Reaction Blank) should appear at the bottom left, close to the origin.



Plot showing heterozygous (green dots), homozygous mut (blue dots) and homozygous wt (red dots) samples for the -13910 C/T polymorphism and a Reaction Blank (black square), using Genvinset® Lactose Intolerance kit Reaction C13910T.



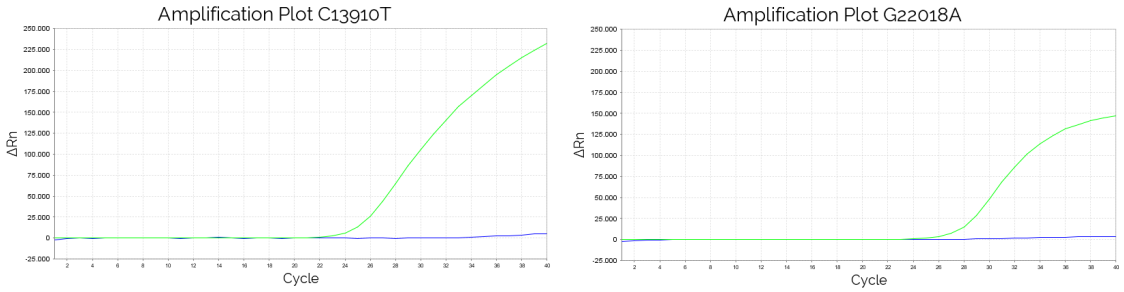
Plot showing heterozygous (green dots), homozygous mut (blue dots) and homozygous wt (red dots) samples for the -22018 G/A polymorphism and a Reaction Blank (black square), using Genvinset® Lactose Intolerance kit Reaction G22018A.

11- Quality control

The kit includes a Reaction Blank, a Control WT (C1) and a Control MUT (C2) which must be tested in both C13910T and G22018A reactions in each assay. An adequate behaviour of these control samples is a guarantee of the proper performance of the reaction.

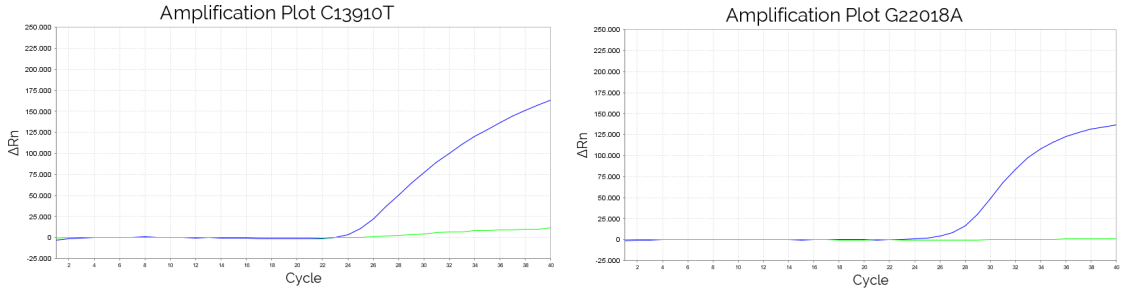
The results are considered valid if the following amplification pattern is obtained in the control samples:

Control WT (C1)



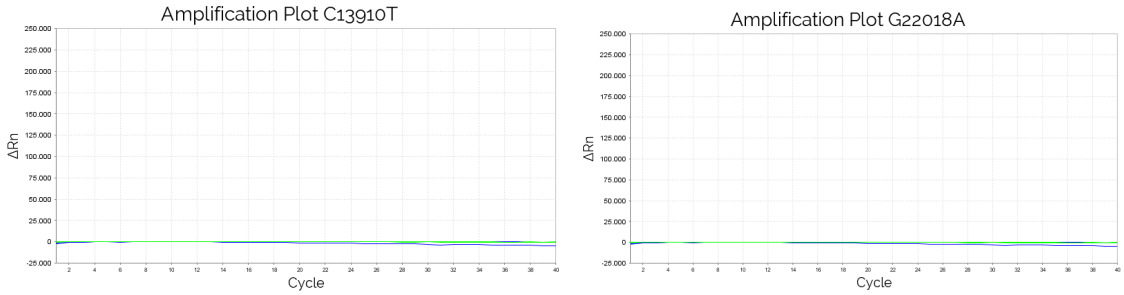
No signal or low intensity signal in FAM channel (blue line) and sigmoidal signal with Ct<35 in HEX channel.

Control MUT (C2)



Amplification signal with Ct<35 in FAM channel (blue line) and no signal or low intensity signal in HEX channel (green line)

Reaction Blank



No signal in both FAM and HEX channels or amplification with Ct>35

The results are considered invalid and should be repeated if:

- An amplification curve with Ct<35 is observed in FAM and/or HEX channels in the Reaction Blank.
- Non-exponential amplification signal is observed or an amplification signal with Ct>35 appears in the Control WT or Control MUT wells.

If adequate behaviour is observed in the positive control reactions, proceed with the interpretation of the rest of the samples as indicated in the previous section.

12- Specific operation data

➔ Analytical specificity

Cross-reactivity has been measured in independent Genvinset® Lactose Intolerance kit validation studies that are described below.

In addition, primers and probes alignment has been checked *in silico*. The alignment of primer sequences is specific. Probes align specifically on the -13910 C/T (NCBI dbSNP rs4988235; NM_005915.6: c.1917+326C>T) and -22018 G/A (NCBI dbSNP rs182549; NM_005915.5: c.1362+117G>A) positions at the *MCM6* gene (OMIM: 601806). No cross-reaction phenomena with genomic DNA have been reported.

Interference has been studied by means of a bibliographic search. Heparin can inhibit Taq polymerase activity and compete with target nucleic acid, so the blood collected must be treated with other anticoagulants as stated in the "Sample collection and preparation" section. Some substances in blood are known to be PCR inhibitors: haemoglobin, hemin, bilirubin, bile salts, lactoferrin, and immunoglobulin G. The polymerase included in Genvinset® Lactose Intolerance has demonstrated high resistance to inhibition, and Master Mix composition is designed to not be affected by interference substances. Nevertheless, potentially inhibitory substances must be removed during DNA extraction and purification protocol. Before delivering results with a diagnostic purpose, a validation assay with such extraction method should be done.

➔ Analytical sensitivity

An assay was performed using 1:4 serial dilutions of three DNA samples, homozygous wild-type (-13910 C/C and -22018 G/G), heterozygous (-13910 C/T and -22018 G/A) and homozygous mutant (-13910 T/T and -22018 A/A) for both C13910T and G22018A polymorphism. DNA samples were obtained by a conventional automated extraction system (QIAsymphony, Qiagen), at a concentration of 76.9, 93.8 and 100.00 ng/µL respectively. The following data were obtained in terms of analytical sensitivity in the detection of wild-type and mutant alleles:

- Detection Limit of wild-type and mutant alleles = 1.5 ng/µL (Ct<35)

➔ Diagnostic sensitivity and specificity

151 DNA samples obtained from several laboratories were analysed. They were previously genotyped by a different kit. The following results were obtained:

Genvinset® Lactose Intolerance									
		C13910T					G22018A		
	Genotype C13910T	C/C	C/T	T/T		Genotype G22018A	G/G	G/A	A/A
Previous method	C/C	51	0	0	Previous method	G/G	48	0	0
	C/T	0	68	0		G/A	0	73	0
	T/T	0	0	32		A/A	0	0	30

There is a 100% match in the results obtained with Genvinset® Lactose Intolerance and the genotyping previously obtained with another commercial kit.

➔ Accuracy

Repeatability study consists in the measurement of within-run variability through the analysis of replicas of every kind of sample that can be measured by the kit (homozygous and heterozygous samples). Each sample was analysed in duplicate.

Genvinset® Lactose Intolerance showed a 100% repeatability.

A study to determine the reproducibility of the reagent was performed. It allowed to estimate the variability between runs, lots, and operators. Samples used represent the full range of expected analyte that can be measured with Genvinset® Lactose Intolerance, that is, homozygous wild-type, homozygous mutant and heterozygous for both C13910T and G22018A polymorphisms. Three operators ran the assay in three different runs and tested three different reagent lots.

Genvinset® Lactose Intolerance showed a 100% reproducibility.

➔ Trueness

The trueness of the analytical procedure of Genvinset® Lactose Intolerance was assessed by comparison with a reference method. The study was developed as an internal validation of the reagent, in which trueness was demonstrated with a 100% value. See "Diagnostic Sensitivity and Specificity" section.

13- Procedure limitations

- The method detects the polymorphisms -13910 C/T (rs4988235) and -22018 G/A (rs182549) at the *MCM6* gene.
- Mutations or polymorphisms at annealing primer/probe sites are possible and may result in the lack of allele definition. Other technologies could be necessary to resolve the typing.
- All the recommendations mentioned in this document should be carefully followed. Any performances that do not meet such indications, can lead to poor results.
- Do not use the kit if there are any suspicions of possible loss of reactivity, contamination, external box deterioration or any other incidence that might affect the kit's performance.
- All Genvinset® reagent manipulations must be done according to good laboratory practice, being adapted to local regulations.
- Do not mix components from other kits or lot numbers.
- Do not use the kit after its expiry date. Dispose of expired reagents according to applicable regulations.
- The real-time PCR thermal cycler must be calibrated according to the manufacturer´s recommendations and should be used accordingly to the manufacturer's instructions.
- Data and result interpretation should be revised by qualified personnel.
- This product is an auxiliary tool for the diagnosis of patients with suspected lactose intolerance. Use these results in conjunction with clinical data and results of other tests performed on the patient.

14- Troubleshooting guide

➔ No amplification signal is detected in any sample (neither in positive controls) or the intensity is very low

- The PCR instrument is not correctly programmed. The thermal profile is not correct/the reading channels are not correctly configured/the selected fluorophores are not suitable.
    - Check that the instrument has been correctly programmed.
- The positions of the samples and controls indicated on the worksheet do not coincide with the positions in which they have been placed in the device.
    - Correctly assign the position of the samples.
- The reagent does not work properly.
    - Ensure that the kit is stored at an appropriate temperature (between -30°C and -18°C) and protected from light. Avoid unnecessary freeze/thaw cycles. Do not use it beyond the expiry date.
- The indicated amounts of each of the reagents have not been added to the reaction mix.
    - Check the volumes of each component added to the mix.
- The consumables used are not compatible with the equipment being used.
    - Make sure that you are using the correct consumables (compatible with the PCR instrument).

➔ No signal is detected in clinical samples (signal appears in positive controls)

- Poor quality of the DNA used.
    - Check the Absorbance ratio 260/280 and discard poor-quality samples. Avoid the presence of inhibitors (heparin, haemoglobin, hemin, bilirubin, bile salts, lactoferrin, immunoglobulin G). Repeat sampling and DNA extraction.
- Inadequate DNA concentration.
    - Check that the DNA concentration is above the limit of detection.
- Inhibition of amplification.
    - Collect whole blood in EDTA or citrate tubes.
- No sample was added.
    - Repeat assay making sure that samples have been added.

➔ Signal detected in the negative control

- Pipetting error.
    - Change the pipette tip each time DNA is added to a well. Check that the sample added to the well corresponds to what is written on the worksheet.
- Contamination of the Primer Mix/Master Mix/Reaction Blank vial.
    - Repeat the assay with fresh aliquots.
- The PCR preparation area is contaminated.
    - Clean surfaces, instruments, lab coats, and change consumables and reagents. Repeat the assay.

➔ Fluorescence intensity varies between samples or abnormal amplification curves are detected

- Dirtiness outside the reaction tube interferes with the detection of fluorescence.

- Clean the equipment thoroughly. Check that the outside of the tubes/plate is clean. Handle the plates/tubes with gloves.

- The volume is not at the bottom of the well or there are bubbles.
  - Centrifuge the plates/tubes before inserting them into the thermal cycler.
  - Check if there are bubbles. If so, remove them.
- The plate/tubes have not been sealed properly.
  - Repeat the assay checking that the plates/tubes have been sealed correctly.
- DNAs with different concentrations have been used or the sample used contains a reaction inhibitor.
  - Repeat the sampling and DNA extraction.
- Presence of polymorphisms or mutations at the probe/primer binding sites.
  - Contact our Technical Support department through *customersupport@bdrdiagnostics.com*.

15- References

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2) Troelsen, J. T. Adult-type hypolactasia and regulation of lactase expression. *Biochim Biophys Acta* **1723**, 19–32 (2005).
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16- Notice to purchaser

- This product has been developed for *in vitro* diagnostic purposes.
- When the kit is used within the territory of any Member State of the European Union, should there be any serious incident regarding the use of the kit, the user must report it to the manufacturer (*regulatory@bdrdiagnostics.com*) and the Competent Authority of their country and/or the patient´s country. Serious incident reporting for all other countries must be conducted according to local requirements for each country.

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17- Changes control

Version	Description of the modification
Rev. 00	First version
Rev. 01	Addition of CE mark and section "Diagnostic sensitivity and specificity"
Rev. 02	Adding CFX96 real-time PCR instrument to the list of validated thermal-cyclers
Rev. 03	Section "Results": graphics correction
Rev. 04	Correction of typos and translation mistakes. New sections: *Accuracy and Trueness*, *Information for safety*. Information regarding intended user, intended patient and interferences has been added. Change in vials volume. Change in Control WT and Control MUT name. Changes on the list of validated real-time PCR cyclers. Insertion of UDI-DI codes.

18- Explanation of symbols used on the labels

IVD	*In vitro* diagnostic medical device		Expiration date
REF	Catalogue number	Σ	Contents sufficient for <n> tests
LOT	Lot number		Manufacturer
	Temperature limit		Keep away from sunlight

CONTROL +	Positive control		Consult electronic Instructions For Use document
CE	This product fulfils the requirements of Directive 98/79/EC on *in vitro* diagnostic medical device		