OCTOBIO

OCTO Health RT-PCR Lactose Intolerance Screening Kit

Catalog #	Description
RT-LAC-25	Real-Time PCR Lactose Intolerance Screening Kit, 25 reactions
RT-LAC-50	Real-Time PCR Lactose Intolerance Screening Kit, 50 reactions

IVD

CE

Introduction

Lactose intolerance is a digestive disorder characterized by the inability to digest lactose, the main carbohydrate in milk and dairy products. This condition results from reduced activity of the enzyme lactase in the small intestine, leading to gastrointestinal symptoms such as bloating, abdominal pain, and diarrhea. The disorder is strongly influenced by genetic variation in the *LCT* gene, as well as regulatory variants in the nearby *MCM* gene, which control whether lactase activity persists or declines after weaning. The incidence of lactose intolerance varies widely, affecting 65–70% of the global population, with especially high rates in East Asian, African, and South American populations. With these rates, lactose intolerance is one of the most widespread food-related metabolic conditions worldwide.

Diagnosis

Diagnosis of lactose intolerance can be performed through clinical and biochemical tests, but genetic analysis offers a reliable and non-invasive method. Variants in the regulatory region of the *MCM6* gene, particularly the SNPs **rs4988235** and **rs182549**, are strongly associated with lactase persistence or non-persistence. Detection of these polymorphisms allows for accurate identification of individuals at risk of lactose intolerance, independent of dietary factors or intestinal health. The OCTO HEALTH REAL TIME PCR LACTOSE INTOLETANCE SCREENING KIT utilizes an RT-PCR instrument combined with melt-curve analysis to detect rs4988235 and rs182549.

Technical Specifications

TUBE	GENE	NUCL. CHANGE	RS #	EMMISION CHANNEL
1	MCM6	c.-13910 C>T	rs4988235	Cy5
	MCM6	c.1362+117 G>A	rs182549	TEXAS RED

Kit Contents

	CONTENT	25 RXN	50 RXN
1	Lactose Intolerance Master Mix	450 µl	900 µl
2	Positive Control*	30 µl	60 µl
3	Negative Control*	30 µl	60 µl

**Positive Control contains synthetic sequence of alternate alleles and Negative Control contains synthetic sequences of reference alleles for each parameter listed in the table, during melt curve analysis, positive control wells should display alternative allele melting temperatures whereas negative control wells should display reference allele melting temperatures.*

Required Equipment and Materials:

- Compatible RT-PCR Devices*

Biorad CFX96 and Opus, Qiagene Rotorgene, Quantstudio Real-Time PCR Systems, Roche LightCycler 480 & 96, Mic qPCR Cycler, Corbett Realtime PCR, Slan Realtime PCR, Molarray MA6000, Himedia Insta Q96, Q48 and Q48M

- DNA Isolation (Manual or automated system)
- Vortex and Centrifuge
- Pipette Set (0.5-10µl and 10-100µl)
- Sterile Pipette Tips
- Cold Block
- PCR tubes or plates compatible with the respective Real-Time PCR Device
- +4°C Refrigerator
- Sterile Gloves

** Please contact for other compatible devices*

Storage Conditions

- All components of the kit should be stored at -20°C until the expiration date.
- Kit components should undergo freeze-thaw cycles a maximum of 5 times. If more than 5 freeze-thaw cycles are anticipated, it is necessary to divide the kit components into smaller volumes for storage.
- Negative and Positive Control included in the kit should be stored at -20 °C after the initial thawing.

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Experimental Preparation

Preparation of Materials:

- Materials stored at -20°C should be thawed on ice or cooling rack.
- Once thawed, liquid materials should be mixed well by pipetting.

Preparation of Reactions:

A single reaction should be prepared for each sample.

Content	Amount (µl)
PCR Master Mix	18
DNA Isolate / NTC	2
TOTAL	20

Real – Time PCR Protocol

Cycle	Temperature	Time	Fluorescence Emission
1	95 °C	300 s	
50	95 °C	10 s	
	60 °C	45 s	TEXAS RED, Cy5
1	95 °C	180 s	
	55 °C	60 s	
Melt Analysis			
1	95 °C	60 s	
1	55 °C	60s	
Initial Temp. (°C)	45 °C	Ramp Rate (°C/sec)	0,3 °C
End Temp. (°C)	75 °C	Hold	2 s

Data Analysis – Interpretation of Results

TUBE 1			
rs4988235 (Cy5)	Reference Allele (C) (62 °C)	Alternative Allele (T) (58 °C)	Result
	+	-	Ref. Homozygous
	-	+	Alt. Homozygous
	+	+	Heterozygous
rs182549 (TEXAS RED)	Reference Allele (G) (63.5 °C)	Alternative Allele (A) (58.5 °C)	Result
	+	-	Ref. Homozygous
	-	+	Alt. Homozygous
	+	+	Heterozygous

- After the Real-Time PCR stage, analysis begins by selecting the Melt Analysis option.
- Each tube must be analyzed separately.
- The analysis should be performed based on the presence or absence of peaks at the specified temperatures for each parameter.

Troubleshooting

1. If no melting peaks are observed in both controls and samples,
   - *Possible causes:* Incomplete homogenization of the master mix; annealing/extension temperature set too high; insufficient or degraded template DNA.
   - *Solutions:* Pipette and briefly centrifuge each reagent tube before setup; verify thermal-cycler temperatures strictly match IFU; repeat DNA extraction and check the quality of extracted DNA by fluorometry.
2. If control or sample melt-curve peaks are markedly lower in fluorescence than expected,
   - *Possible causes:* Dye depletion due to ambient light exposure or prolonged room-temperature storage; expired or improperly stored reagents.
   - *Solutions:* Protect master mix and reaction plates from light and keep on ice until use; check kit lot's expiration and storage logs; replace with a fresh kit if needed.
3. If melt peaks appear at temperatures outside the ±1 °C window of the expected T_m,
   - *Possible causes:* Incorrect ramp rate or hold time during melt acquisition; nonspecific amplicons (e.g. primer-dimers).
   - *Solutions:* Confirm melt-curve program parameters exactly match IFU.
4. If melt-curve derivative baselines are noisy or drift upward,
   - *Possible causes:* Insufficient calibration of the instrument's optical modules; wells at plate edges subject to temperature lag.
   - *Solutions:* Perform calibration with manufacturer's reference plate; avoid using outermost wells for critical controls or include a software-specified "edge-correction."
5. If sample genotyping calls fail QC despite clean control results,
   - *Possible causes:* Sample inhibitors (e.g. heme, guanidine) carried over from extraction; pipetting errors.
   - *Solutions:* Repeat extraction using a spin-column kit with an inhibitor-removal step; employ multichannel pipette with filtered tips and include replicate wells.
6. If unexpected peaks appear in the no-template control (NTC),
   - *Possible causes:* Airborne DNA contamination.
   - *Solutions:* Enforce unidirectional workflow (pre-PCR vs. post-PCR areas).

Tip: Always consult your instrument's maintenance log and ensure that filter sets and heating blocks are within specification before attributing anomalies to the chemistry.

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Warnings and Precautions

This test is for in vitro diagnostic purposes only and should be used strictly in accordance with the instructions provided by trained personnel. Clinical samples should be considered as materials potentially carrying an infection risk and should be prepared in a laminar flow cabinet.

• Do not use expired kit. • Avoid repeated freezing and thawing of reagents as it may reduce the sensitivity of the test. • Ensure that reagents are thawed before use, then gently mix the tubes and centrifuge briefly. • Prepare the reaction mixture quickly on ice or a cold block. • Establish two separate work areas, one for isolation and the other for PCR. • Avoid transferring pipettes, tubes, and other working materials between the work areas. • Always use filtered sterile pipette tips. • Use separate lab coats and gloves for each work area.

Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard **TS EN ISO 15223-1**



: Number of Reactions


: Expiration Date



: Lot Number



: Reference Number


: Temperature Limit


: Refer to the User Manual


: Manufacturer


: Keep Away From Direct Sunlight

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