CELIAC REAL TIME PCR KIT

(HLA-DQ2, HLA-DQ8)

Cat. No:111R-20-04

PRODUCT DESCRIPTION

Celiac disease is a genetically determined immune-mediated disorder, that develops in genetically susceptible individuals mainly as a result of enteric exposure to gluten proteins after digestion of wheat, barley and rye. The genetic risk factors for celiac disease have been well characterized. More than 90% of the patients carry a major histocompatibility complex class II human leukocyte antigen (HLA) variant called DQ2 (encoded by DQB1*02 and DQA1*05). Most of the remainder carry an HLA variant called DQ8 (encoded by DQB1*0302 and DQA1*03). DQ2 and DQ8 variants are necessary for the development of celiac disease.

PRINCIPLE OF THE SYSTEM

During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencer dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

PRODUCT SPECIFICATION

Each isolated DNA should be tested with Mix 1 and Mix 2. The kit provides reagents in a ready-to-use mastermix format which has been specifically adapted for 5' nuclease PCR. The test system is designed for use with sequence specific primers and probe.

The fluorescences of mutation analysis are FAM and Texas Red. Also each mastermix contains an internal control labelled with HEX/JOE dye.

The limit of detection (LOD) for the Celic Real Time PCR Kit was determined as ≤ 1%.

SYSTEM CONTENTS

Reagents	20 rxns
• Celiac Mix 1	400 µl
• Celiac Mix 2	400 µl
• Control DNA	30 µl

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.

DNA EXTRACTION

Blood samples should be collected in appropriate sterile EDTA tubes and can be stored at +4°C up to one month. For more than one month specimen should be stored at -20°C. It is advised to gently mix the tube (with EDTA) after collection of blood to avoid coagulation.

Our system optimized according to SNPure Blood® and MN NucleoSpin ® Blood. It is advised to elute DNA with **150 µl elution buffer** for better results.

MUTATION / DYE TABLE

Table 1 : Tubes- mutations- dyes.

Tubes	Mutations	Dyes
Mix 1	DQB1*02	FAM
	DQA1*05	TEXAS RED
	Internal Control	HEX/JOE
Mix 2	DQB1*0302	FAM
	DQA1*03	TEXAS RED
	Internal Control	HEX/JOE

PROCEDURE

- Different tubes should be prepared for each mix.
- Before starting work, mix the mastermixes gently by pipetting
- For each sample, pipet **20 µl mastermix*** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl DNA** into each tube**.**
- Run with the programme shown below.

Master mixes include HotStart Taq DNA Polymerase.

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	30 Cycles
60 °C	1 Min.	

Fluorescent dyes are FAM, TEXAS RED and HEX/JOE.

This system can use with;

ABI Prism ® 7500/7500 Fast*

Bio-Rad CFX96

***If you use;**

- ABI Prism® system, please choose **"none"** as passive reference and quencher.

DATA ANALYSIS

After the run is completed data are analysed using the software with HEX/JOE, TEXAS RED and FAM dyes. The below results were studied with Bio-Rad CFX96.

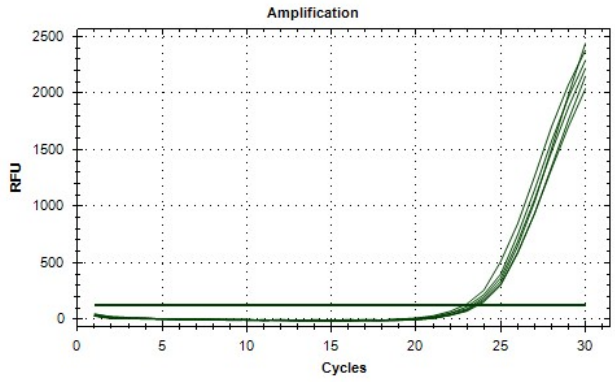

Figure 1: Internal Control plots – HEX/JOE Dye

Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX/JOE dye. The CT value of internal controls should be **21 ≤ X ≤ 26**.

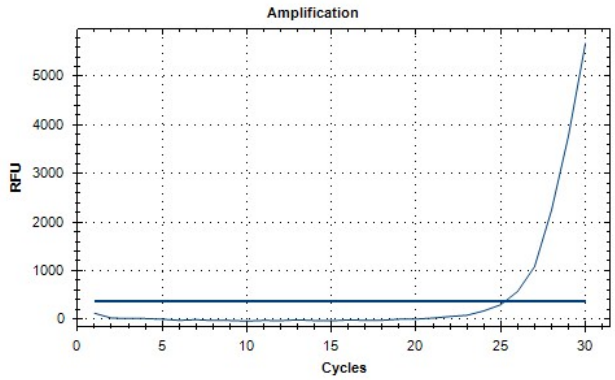

Figure 2: DQB1*02 Positive result – FAM Dye

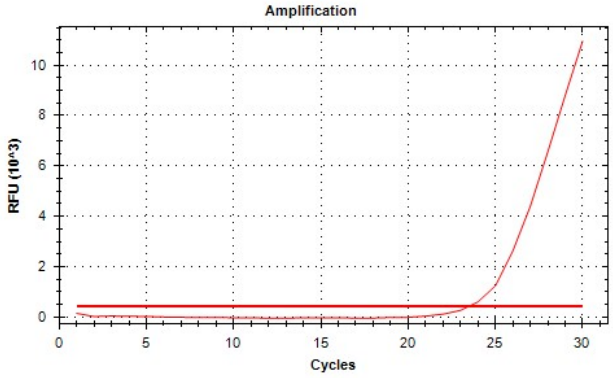

Figure 3: DQA1*05 Positive result – TEXAS RED Dye

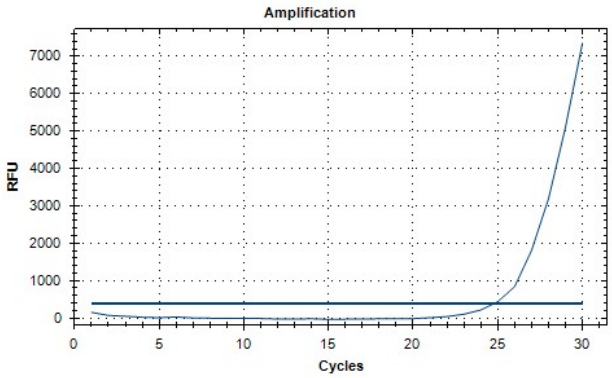

Figure 4: DQB1*0302 Positive result – FAM Dye

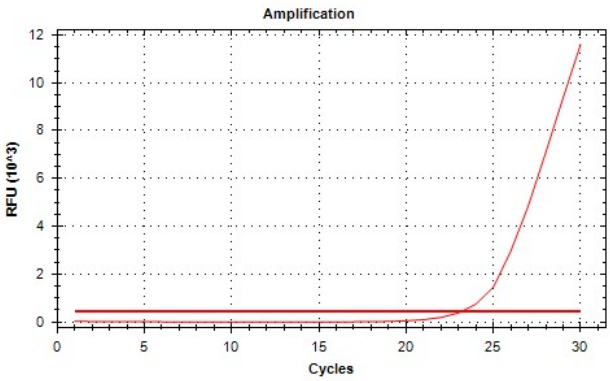

Figure 5: DQA1*03 Positive result – TEXAS RED Dye

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Absence/Deficiency of Hot Start Taq DNA Polymerase
- Sample is containing DNA inhibitor(s)

If plots start late,

- DNA quality is not good.
- The amount of DNA is not enough.
- Sample is containing parcial DNA inhibitor(s)

Please contact us for your questions. tech@snp.com.tr

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR mastermixes forgotten at room temperature.
- Thaw PCR mastermix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR mastermix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.