CONNEXIN MULTIPLEX REAL TIME PCR KIT (10 MUTATIONS)

Cat. No: 100R-20-10

PRODUCT DESCRIPTION

Congenital deafness is inherently an autosomal recessive disease. 98% of the mutations that cause this disease occur in the Connexin 26 gene (GJB2). Kit can detect ten mutations on this gene; 35DelG, 167delT, M34T, L90P, R184P (G>C), V37I, IVS 1+1 G>A, W24X, 312Del and E47X.

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 wild type and mutant real time pcr mastermixes. The kit provides reagents in a ready-to-use mastermix format which has been specifically adapted for 5' nuclease PCR using SNP analyses. The test system is designed to use with sequence specific primers and probe.

The fluorescence of mutation analysis is FAM and HEX/JOE. Also each mastermix contains an internal control labelled with CY5 dye. Mutations and related dyes can be seen in Table 1.

SYSTEM CONTENTS

Reagents	20 rxns
• Con Mix 1	400 µl
• Con Mix 2	400 µl
• Con Mix 3	400 µl
• Con Mix 4	400 µl
• Con Mix 5	400 µl
• Con Mix 6	400 µl
• Con Mix 7	400 µl
• Con Mix 8	400 µl
• Con Mix 9	400 µl
• Con Mix 10	400 µl
• Control DNA	75 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	35DelG Wild-Type	FAM
	167Del Wild-Type	JOE / HEX
	Internal Control	CY5
Mix 2	35DelG Mutant	FAM
	167Del Mutant	JOE / HEX
	Internal Control	CY5
Mix 3	M34T Wild-Type	FAM
	L90P Wild-Type	JOE / HEX
	Internal Control	CY5
Mix 4	M34T Mutant	FAM
	L90P Mutant	JOE / HEX
	Internal Control	CY5
Mix 5	R184P Wild-Type	FAM
	V37I Wild-Type	JOE / HEX
	Internal Control	CY5
Mix 6	R184P Mutant	FAM
	V37I Mutant	JOE / HEX
	Internal Control	CY5
Mix 7	IVS1+1 Wild-Type	FAM
	V24X Wild-Type	JOE / HEX
	Internal Control	CY5
Mix 8	IVS1+1 Mutant	FAM
	V24X Mutant	JOE / HEX
	Internal Control	CY5
Mix 9	312Del Wild-Type	FAM
	E47X Wild-Type	JOE / HEX
	Internal Control	CY5
Mix 10	312Del Mutant	FAM
	E47X Mutant	JOE / HEX
	Internal Control	CY5

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 (~10-100 ng) 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, HEX/JOE and CY5.

If you use;

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

This system can be used with;

Bio-Rad CFX96

ABI Prism ® 7500/7500 Fast

Roche LightCycler® 480 System

DATA ANALYSIS

After the run is completed data are analysed using the software with FAM, HEX (JOE) and CY5 dyes. The below results were studied with ABI 7500.

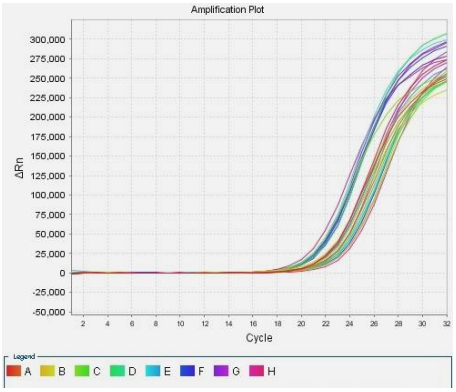

Figure 1: Internal Control plots – CY5 Dye

Internal control amplification plots must be seen in all wells except NTC and has been labelled with CY5 dye. The CT value of internal controls should be **21 ≤ X ≤ 26**.

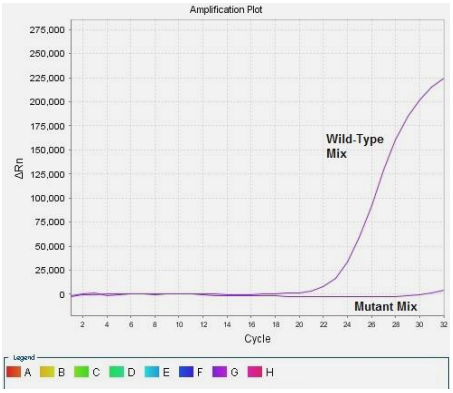

Figure 2: 35DelG Wild Type – FAM Dye (Mix 1 – 2)

Amplification plots of mutations can be analysed by related dye*. The CT value should be between **21 ≤ CT ≤ 26**. These values are optimised according to the SNPure® Blood DNA Isolation Kıt and MN NucleoSpin ® Blood DNA Isolation Kıt. CT values may vary ±2/3 cycle according to the DNA isolation protocol.

- Homozygote wild-type sample gives amplification signal only with wild-type mastermix.
- Heterozygote sample gives amplification signal both with wild-type and mutant mastermixes.
- Homozygote mutant sample gives amplification signal only with mutant mastermix.
- The diffrence of the CT value wild-type and mutant amplification plots should be **≤3** for heterozygote mutant sample. It is **4 ≤ CT ≤6**, test should be repeated.

**Please check tubes / mutations / dyes table (table 1).*

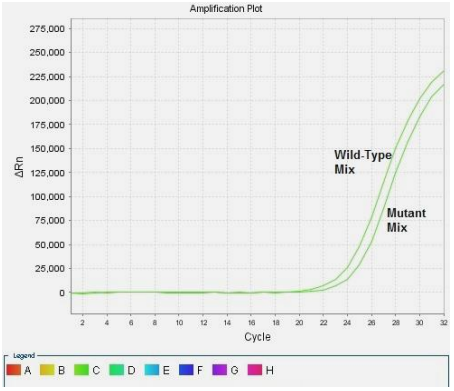

Figure 3: L90P Heterozygote – JOE/HEX Dye (Mix 3 – 4)

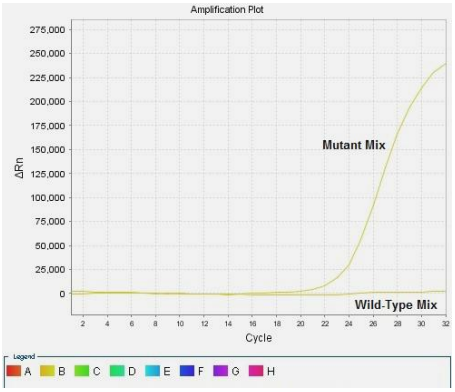

Figure 4: IVS1+1 Homozygote mutant – FAM Dye (Mix 7 – 8)

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- 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.