

CYSTIC FIBROSIS (CF) MULTIPLEX REAL TIME PCR KIT (18 MUTATIONS)

Cat. No: 14R-20-18

PRODUCT DESCRIPTION

Cystic fibrosis (CF) is a common hereditary disease which affects the entire body, causing progressive disability and often early death. Difficulty in breathing is the most serious symptom and results from frequent lung infections that are treated, though not cured, by antibiotics and other medications. CF is caused by a mutation in the gene for the protein cystic fibrosis transmembrane conductance regulator (CFTR). This gene is required to regulate the components of sweat, digestive juices, and mucus. Although most people without CF have two working copies of the CFTR gene, only one is needed to prevent cystic fibrosis. CF develops when neither gene works normally.

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 quencher 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, HEX/JOE and Texas Red. 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
• CF-Mix 1	400 µl
• CF-Mix 2	400 µl
• CF-Mix 3	400 µl
• CF-Mix 4	400 µl
• CF-Mix 5	400 µl
• CF-Mix 6	400 µl
• CF-Mix 7	400 µl
• CF-Mix 8	400 µl
• CF-Mix 9	400 µl
• CF-Mix 10	400 µl
• CF-Mix 11	400 µl
• CF-Mix 12	400 µl
• Control DNA	75 µl

Table 1 : Tubes- mutations- dyes.

Tubes	Mutations	Dyes
Mix 1	G542X Wild Type	FAM
	W1282X Wild Type	JOE / HEX
	E92K Wild Type	Texas Red
	Internal Control	CY5
Mix 2	G542X Mutant Type	FAM
	W1282X Mutant Type	JOE / HEX
	E92K Mutant Type	Texas Red
	Internal Control	CY5
Mix 3	1677delTA Wild Type	FAM
	K68E Wild Type	JOE / HEX
	2789+5G>A Wild Type	Texas Red
	Internal Control	CY5
Mix 4	1677delTA Mutant Type	FAM
	K68E Mutant Type	JOE / HEX
	2789+5G>A Mutant Type	Texas Red
	Internal Control	CY5
Mix 5	ΔF508 Wild Type	FAM
	1717-1G>A Wild Type	JOE / HEX
	4010delTATT Wild Type	Texas Red
	Internal Control	CY5
Mix 6	ΔF508 Mutant Type	FAM
	1717-1G>A Mutant Type	JOE / HEX
	4010delTATT Mutant Type	Texas Red
	Internal Control	CY5
Mix 7	F1052V Wild Type	FAM
	I148T Wild Type	JOE / HEX
	G551D Wild Type	Texas Red
	Internal Control	CY5
Mix 8	F1052V Mutant Type	FAM
	I148T Mutant Type	JOE / HEX
	G551D Mutant Type	Texas Red
	Internal Control	CY5
Mix 9	Y1032C Wild Type	FAM
	3849+10kbC>T Wild Type	JOE / HEX
	R560T Wild Type	Texas Red
	Internal Control	CY5
Mix 10	Y1032C Mutant Type	FAM
	3849+10kbC>T Mutant Type	JOE / HEX
	R560T Mutant Type	Texas Red
	Internal Control	CY5
Mix 11	2183AA--G Wild Type	FAM
	S4X Wild Type	JOE / HEX
	N1303K Wild Type	Texas Red
	Internal Control	CY5
Mix 12	2183AA--G Mutant Type	FAM
	S4X Mutant Type	JOE / HEX
	N1303K Mutant Type	Texas Red
	Internal Control	CY5

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 MN NucleoSpin® Blood. It is advised to elute DNA with **150 µl elution buffer** for better results.

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	5 Sec.	32 Cycles
60 °C	45 Sec.	

Fluorescent dyes are FAM, TEXAS RED, CY5 and HEX/JOE.

This system can use with:

ABI 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 FAM, HEX (JOE), TEXAS RED and CY5 dyes. The below results were studied with BioRad CFX96.

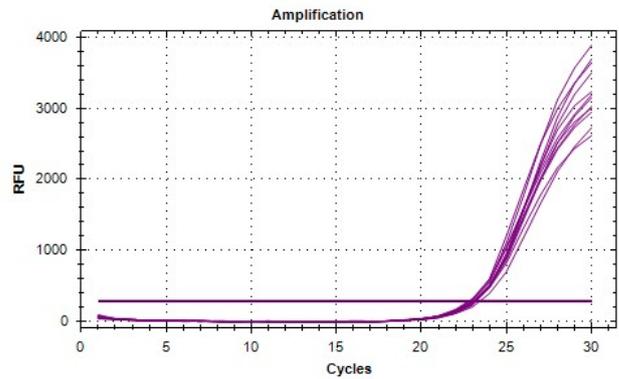


Figure 1: Internal control – CY5 Dye

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 $22 \leq X \leq 26$.

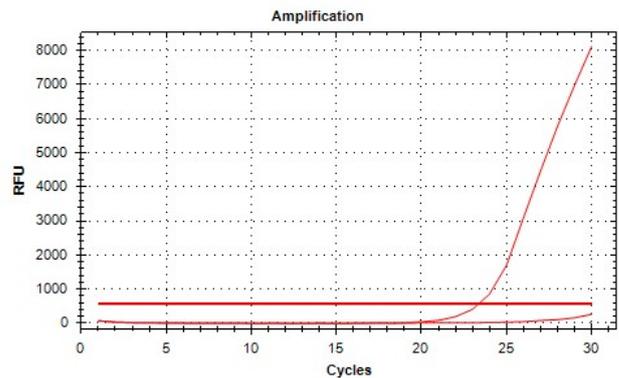


Figure 2: 2789+5G>A Wild Type (Mix 3 & 4) – TEXAS RED Dye

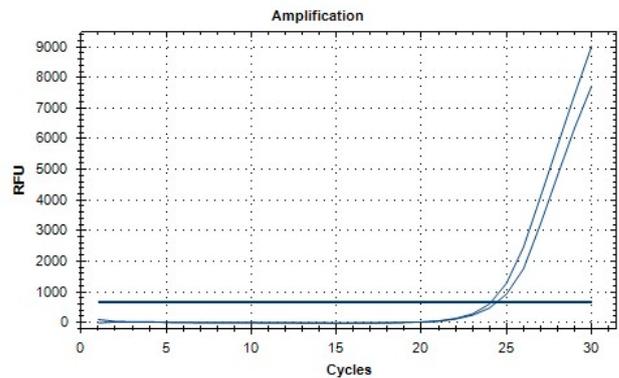


Figure 3: ΔF508 Heterozygote (Mix 5 & 6) – FAM Dye

Amplification plots of mutations can be analysed by related dye*. The CT value should be between $21 \leq CT \leq 26$. These values are optimised according to the SNPure® Blood DNA Isolation Kit and MN NucleoSpin® Blood DNA Isolation Kit. CT values may vary $\pm 2/3$ 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 difference of the CT value wild-type and mutant amplification plots should be ≤ 3 for heterozygote mutant sample. It is $4 \leq CT \leq 6$, test should be repeated.

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

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 ($>3X$) should be avoided, as this may reduce the sensitivity of the assay.

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Sample is containing DNA inhibitor(s)

If plots start late,

Compare positive control and sample. If there is no problem in positive control,

- DNA quality is not good.
- The amount of DNA is not enough.

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.

Well	Sample	Mix1 (WT)	Mix2 (MT)	Mix3 (WT)	Mix4 (MT)	Mix5 (WT)	Mix6 (MT)	Mix7 (WT)	Mix8 (MT)	Mix9 (WT)	Mix10 (MT)	Mix11 (WT)	Mix12 (MT)
A	G542X / FAM		1677delTAl / FAM		ΔF508 / FAM		F1052V / FAM		Y1032C / FAM		2183AA-G / FAM		
	W1282X / JOE-HEX		K68E / JOE-HEX		1717-1G>Al / JOE-HEX		I148T / JOE-HEX		3849+100bC>T / JOE-HEX		S4X / JOE-HEX		
	E92K / Texas Red		Z789+5G>A / Texas Red		4010delTATT / Texas Red		G551D / Texas Red		R560T / Texas Red		N1303K / Texas Red		
	Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		
B	G542X / FAM		1677delTAl / FAM		ΔF508 / FAM		F1052V / FAM		Y1032C / FAM		2183AA-G / FAM		
	W1282X / JOE-HEX		K68E / JOE-HEX		1717-1G>Al / JOE-HEX		I148T / JOE-HEX		3849+100bC>T / JOE-HEX		S4X / JOE-HEX		
	E92K / Texas Red		Z789+5G>A / Texas Red		4010delTATT / Texas Red		G551D / Texas Red		R560T / Texas Red		N1303K / Texas Red		
	Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		
C	G542X / FAM		1677delTAl / FAM		ΔF508 / FAM		F1052V / FAM		Y1032C / FAM		2183AA-G / FAM		
	W1282X / JOE-HEX		K68E / JOE-HEX		1717-1G>Al / JOE-HEX		I148T / JOE-HEX		3849+100bC>T / JOE-HEX		S4X / JOE-HEX		
	E92K / Texas Red		Z789+5G>A / Texas Red		4010delTATT / Texas Red		G551D / Texas Red		R560T / Texas Red		N1303K / Texas Red		
	Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		
D	G542X / FAM		1677delTAl / FAM		ΔF508 / FAM		F1052V / FAM		Y1032C / FAM		2183AA-G / FAM		
	W1282X / JOE-HEX		K68E / JOE-HEX		1717-1G>Al / JOE-HEX		I148T / JOE-HEX		3849+100bC>T / JOE-HEX		S4X / JOE-HEX		
	E92K / Texas Red		Z789+5G>A / Texas Red		4010delTATT / Texas Red		G551D / Texas Red		R560T / Texas Red		N1303K / Texas Red		
	Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		
E	G542X / FAM		1677delTAl / FAM		ΔF508 / FAM		F1052V / FAM		Y1032C / FAM		2183AA-G / FAM		
	W1282X / JOE-HEX		K68E / JOE-HEX		1717-1G>Al / JOE-HEX		I148T / JOE-HEX		3849+100bC>T / JOE-HEX		S4X / JOE-HEX		
	E92K / Texas Red		Z789+5G>A / Texas Red		4010delTATT / Texas Red		G551D / Texas Red		R560T / Texas Red		N1303K / Texas Red		
	Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		
F	G542X / FAM		1677delTAl / FAM		ΔF508 / FAM		F1052V / FAM		Y1032C / FAM		2183AA-G / FAM		
	W1282X / JOE-HEX		K68E / JOE-HEX		1717-1G>Al / JOE-HEX		I148T / JOE-HEX		3849+100bC>T / JOE-HEX		S4X / JOE-HEX		
	E92K / Texas Red		Z789+5G>A / Texas Red		4010delTATT / Texas Red		G551D / Texas Red		R560T / Texas Red		N1303K / Texas Red		
	Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		
G	G542X / FAM		1677delTAl / FAM		ΔF508 / FAM		F1052V / FAM		Y1032C / FAM		2183AA-G / FAM		
	W1282X / JOE-HEX		K68E / JOE-HEX		1717-1G>Al / JOE-HEX		I148T / JOE-HEX		3849+100bC>T / JOE-HEX		S4X / JOE-HEX		
	E92K / Texas Red		Z789+5G>A / Texas Red		4010delTATT / Texas Red		G551D / Texas Red		R560T / Texas Red		N1303K / Texas Red		
	Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		
H	G542X / FAM		1677delTAl / FAM		ΔF508 / FAM		F1052V / FAM		Y1032C / FAM		2183AA-G / FAM		
	W1282X / JOE-HEX		K68E / JOE-HEX		1717-1G>Al / JOE-HEX		I148T / JOE-HEX		3849+100bC>T / JOE-HEX		S4X / JOE-HEX		
	E92K / Texas Red		Z789+5G>A / Texas Red		4010delTATT / Texas Red		G551D / Texas Red		R560T / Texas Red		N1303K / Texas Red		
	Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		Int. Control / CV5		