BETA THALASSEMIA MULTIPLEX REAL TIME PCR KIT (12 MUTATIONS)
Cat. No: 16R-20-12

PRODUCT DESCRIPTION

Beta Thalassemia is a group of inherited autosomal recessive blood disorders that originated in the Mediterranean region. In thalassemia the genetic defect, which could be either mutation or deletion, results in reduced rate of synthesis or no synthesis of one of the globin chains that make up hemoglobin. This can cause the formation of abnormal hemoglobin molecules, thus causing anemia, the characteristic presenting symptom of the thalassemias. Beta Thalassemia Real Time PCR Kit includes, IVS1.1 (G>A), IVS2.1 (G>A), IVS1.110 (G>A), IVS2.745 (C>G), IVS1.5 (G>C), IVS1.6 (T>C), -30(T>A), Cd 5 (-CT), Cd 29 (C>T), Cd 39 (C>T), Cd 44 (-C) and HBS mutations.

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 12 mixes. The kit provides reagents in a ready-to-use mastermix format which has been specifically adapted for 5' nuclease PCR using patented SNP analyses. The test system is designed for use with sequence specific primers and probe.

The fluorescence of mutation analysis is FAM, HEX/JOE. Also each mastermix contains an internal control labelled with CY5 dye.

SYSTEM CONTENTS

Reagents	20 Rxns
• BT-Mix 1	400 µl
• BT-Mix 2	400 µl
• BT-Mix 3	400 µl
• BT-Mix 4	400 µl
• BT-Mix 5	400 µl
• BT-Mix 6	400 µl
• BT-Mix 7	400 µl
• BT-Mix 8	400 µl
• BT-Mix 9	400 µl
• BT-Mix 10	400 µl
• BT-Mix 11	400 µl
• BT-Mix 12	400 µl
• Control DNA*	75 µl

*Control DNA is a synthetic plasmid containing some of the mutation regions. Expected results for synthetic control DNA should be IVS1.6 Heterozygous, -30(T>A) Homozygote Mutant, Cd 5 Wild Type, IVS1.110 Wild Type, Cd 39 Wild Type, HBS Wild Type, Cd 44 Wild Type, IVS2.1 Homozygote Mutant, IVS1.5 Homozygote Mutant. Amplification plots of synthetic control DNA may appear slightly different from the sample DNA.

Table 1 : Tubes- mutations- dyes.

Tubes	Mutations	Dyes
Mix 1	IVS1.6 (T>C) Wild Type	FAM
	-30(T>A) Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 2	IVS1.6 (T>C) Mutant Type	FAM
	-30(T>A) Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 3	Cd 5 (-CT) Wild Type	FAM
	IVS1.110 (G>A) Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 4	Cd 5 (-CT) Mutant Type	FAM
	IVS1.110 (G>A) Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 5	Cd 39 (C>T) Wild Type	FAM
	HBS Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 6	Cd 39 (C>T) Mutant Type	FAM
	HBS Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 7	Cd 29 (C>T) Wild Type	FAM
	Cd 44 (-C) Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 8	Cd 29 (C>T) Mutant Type	FAM
	Cd 44 (-C)Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 9	IVS2.1 (G>A) Wild Type	FAM
	IVS1.1 (G>A) Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 10	IVS2.1 (G>A) Mutant Type	FAM
	IVS1.1 (G>A) Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 11	IVS2.745 (C>G) Wild Type	FAM
	IVS1.5 (G>C) Wild Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5
Mix 12	IVS2.745 (C>G) Mutant Type	FAM
	IVS1.5 (G>C) Mutant Type	JOE / HEX
	Empty	Texas Red
	Internal Control	CY5

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.

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

Table 2 : 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.

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) and CY5 dyes. The below results were studied with BioRad CFX96.
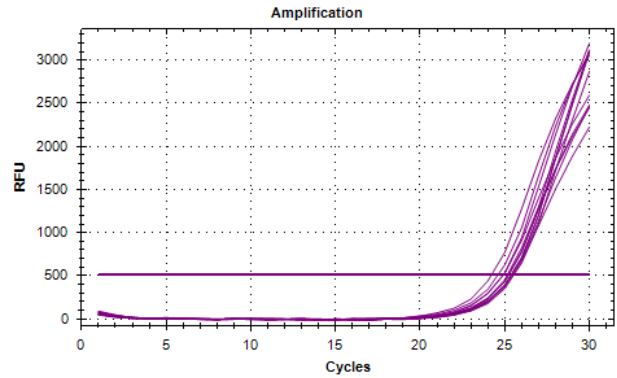
Figure 1: Internal control – CY5 Dye

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 ≤ X ≤ 26**.
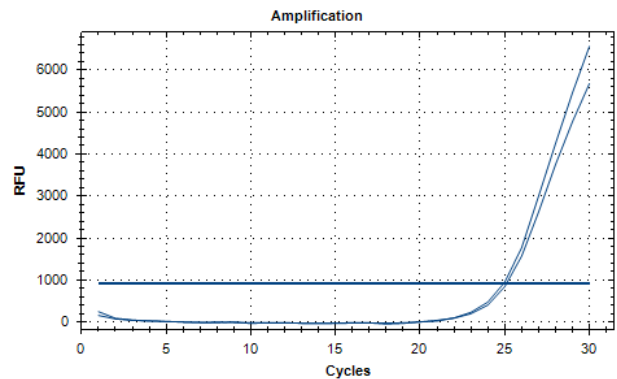
Figure 2: IVS2.1 G>A Heterozygote (Mix 9 & 10) – FAM Dye

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).
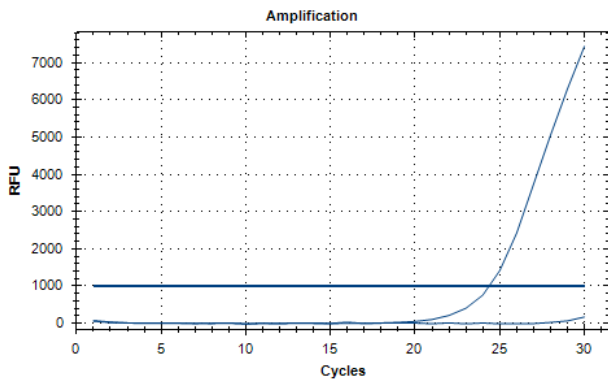
Figure 3: IVS2.745 C>G Wild Type (Mix 11 & 12) – FAM Dye
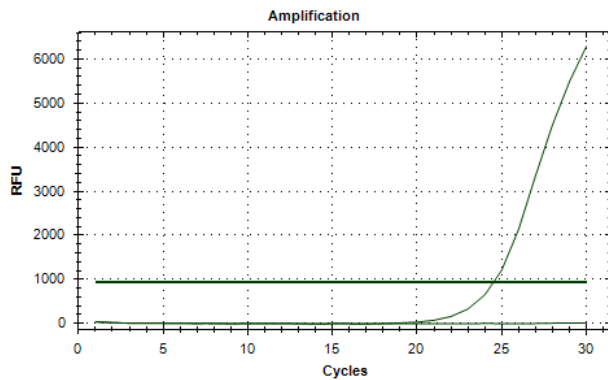
Figure 4: IVS1.110 G>A Wild Type (Mix 3 & 4) – HEX Dye

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	FAM / JOE-HEX / CY5	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		FAM	IVS1.6 (T>C)	Cd 5 (-CT)	Cd 39 (C>T)	Cd 29 (C>T)	IVS2.1 (G>A)	IVS2.745 (C>G)
		JOE-HEX	-30(T>A)	IVS1.110 (G>A)	HBS	Cd 44 (-C)	IVS1.1 (G>A)	IVS1.5 (G>C)
		CY5	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control
B		FAM	IVS1.6 (T>C)	Cd 5 (-CT)	Cd 39 (C>T)	Cd 29 (C>T)	IVS2.1 (G>A)	IVS2.745 (C>G)
		JOE-HEX	-30(T>A)	IVS1.110 (G>A)	HBS	Cd 44 (-C)	IVS1.1 (G>A)	IVS1.5 (G>C)
		CY5	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control
C		FAM	IVS1.6 (T>C)	Cd 5 (-CT)	Cd 39 (C>T)	Cd 29 (C>T)	IVS2.1 (G>A)	IVS2.745 (C>G)
		JOE-HEX	-30(T>A)	IVS1.110 (G>A)	HBS	Cd 44 (-C)	IVS1.1 (G>A)	IVS1.5 (G>C)
		CY5	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control
D		FAM	IVS1.6 (T>C)	Cd 5 (-CT)	Cd 39 (C>T)	Cd 29 (C>T)	IVS2.1 (G>A)	IVS2.745 (C>G)
		JOE-HEX	-30(T>A)	IVS1.110 (G>A)	HBS	Cd 44 (-C)	IVS1.1 (G>A)	IVS1.5 (G>C)
		CY5	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control
E		FAM	IVS1.6 (T>C)	Cd 5 (-CT)	Cd 39 (C>T)	Cd 29 (C>T)	IVS2.1 (G>A)	IVS2.745 (C>G)
		JOE-HEX	-30(T>A)	IVS1.110 (G>A)	HBS	Cd 44 (-C)	IVS1.1 (G>A)	IVS1.5 (G>C)
		CY5	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control
F		FAM	IVS1.6 (T>C)	Cd 5 (-CT)	Cd 39 (C>T)	Cd 29 (C>T)	IVS2.1 (G>A)	IVS2.745 (C>G)
		JOE-HEX	-30(T>A)	IVS1.110 (G>A)	HBS	Cd 44 (-C)	IVS1.1 (G>A)	IVS1.5 (G>C)
		CY5	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control
G		FAM	IVS1.6 (T>C)	Cd 5 (-CT)	Cd 39 (C>T)	Cd 29 (C>T)	IVS2.1 (G>A)	IVS2.745 (C>G)
		JOE-HEX	-30(T>A)	IVS1.110 (G>A)	HBS	Cd 44 (-C)	IVS1.1 (G>A)	IVS1.5 (G>C)
		CY5	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control
H		FAM	IVS1.6 (T>C)	Cd 5 (-CT)	Cd 39 (C>T)	Cd 29 (C>T)	IVS2.1 (G>A)	IVS2.745 (C>G)
		JOE-HEX	-30(T>A)	IVS1.110 (G>A)	HBS	Cd 44 (-C)	IVS1.1 (G>A)	IVS1.5 (G>C)
		CY5	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control	Int. Control