

## Quantiplus® Beta thalassemia Qualitative PCR Kit (Sequencing based PCR Kit)



SEQ-βTH-25 : 25 rxns  
 SEQ-βTH-50 : 50 rxns  
 SEQ-βTH-100 : 100 rxns

RUO

PI/ SEQβTH -00

### Introduction

Beta thalassemia is a blood disorder that reduces the production of hemoglobin. Hemoglobin is the iron-containing protein in red blood cells that carries oxygen to cells throughout the body. In people with Beta thalassemia, low levels of hemoglobin lead to lack of oxygen in many parts of the body. This test is used to investigate deletions and mutations in the alpha- and beta-globin producing genes. Family studies can be done to evaluate carrier status and the types of mutations present in other family members.

### Product Description

Quantiplus® Beta Thalassemia PCR sequencing kit, is an *in vitro* nucleic acid amplification test based on conventional qualitative PCR and DNA sequencing for the detection of sickle cell and β-Globin mutations [FS 8/9 (+g), Codon 15 (g>a), Codon 30 (g>a), IVS1-1 (g>t), IVS1-5 (g>c), FS 41/42 (-cttt), Hbs (g>a), HbE (g>a)], in human DNA. The amplified products then have to be purified from Agarose gel followed by automated DNA sequencing.

### Kit components

Color Coding (Caps)	Contents	Description	25 rxns (SEQ-βTH-25)	50 rxns (SEQ-βTH-50)	100 rxns (SEQ-βTH-100)
Blue	Universal PCR Mix(2X)	DNA Amplification Reagent	1 x 315 μL	1 x 625 μL	2 x 625 μL
Amber	HUWEL β-Thal PM	Primer mix	1 x 50 μL	1 x 100 μL	2 x 100 μL
Natural	HUWEL β-Thal SPF	Forward Sequencing Primer	1 x 25 μL	1 x 50 μL	1 x 100 μL
Natural	HUWEL β-Thal SPR	Reverse Sequencing Primer	1 x 25 μL	1 x 50 μL	1 x 100 μL
Pink	Amplification Control	Amplification Control	1 x 25 μL	1 x 25 μL	1 x 25 μL
White	MBGPW	Purified water	1 x 500 μL	1 x 500 μL	1 x 1mL

### Storage and Transportation Conditions

The kits could be transported at temperature below -20 °C. The kit will remain stable at least until the expiry date printed on the package, if the storage temperature is kept (-20 ± 5 °C). Kit is stable after 4 repeated freezing/thawing cycles. The reagents should be frozen in aliquots, if they are to be used intermittently.

**Technical Specification**

Target Sequence	8 common mutations [FS 8/9 (+g), Codon 15 (g>a), Codon 30 (g>a), IVS1-1 (g>t), IVS1-5 (g>c), FS 41/42 (-cttt), Hbs (g>a), HbE (g>a)]
Specificity	100% specificity
Validated Specimen	K2EDTA Blood

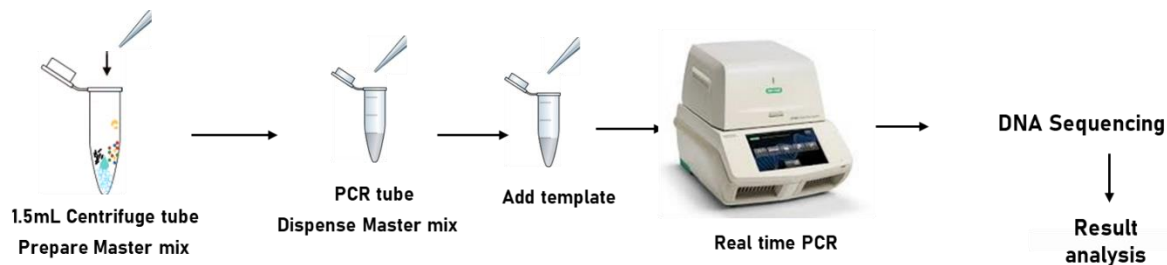
**Assay Procedure****DNA Extraction**

Quantiplus® Beta Thalassemia Qualitative PCR Kit has been validated using the following DNA extraction kits:

Recommended sample volume for extraction and elution are as follows:

S. No.	Name of the Extraction Kit	Recommended Sample volume for Extraction	Recommended Final Elution volume
1.	Huwel Nucleic Acid Extraction Kit - Version 2.0 (Cat. No. HL-NAX-100)	200 µL	100 µL

Note: Customer can also validate their own extraction process using other DNA extraction Kits.

**qPCR Protocol Flow****Preparation of Reaction Master mix**

Components	Volume per reaction (For 25µL)
Universal PCR Mix(2x)	12.5
HUWEL β-thal Primer Mix	2.0
MBGPW	5.5
Extracted DNA / Amplification Control/MBGPW	5.0

It is necessary to keep all components at +2 °C to +8 °C during the PCR preparation. Close the tubes, centrifuge briefly before processing to thermal cycler.

**Cycling Conditions**

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	5 min
2 (PCR cycling)	35	94	30 sec
		57	30 sec
		72	40 sec
		72	5 min
3 (Final Extension)	1	72	5 min
4 (Reaction Hold)	1	4	Hold

### **Gel Electrophoresis**

The amplified PCR products are resolved on 2% Agarose gel in 1X TAE or 1X TBE buffer along with 100 bp DNA ladder for size determination and result interpretation.

### **Sample analysis and Interpretation**

Analyze the amplified DNA on 2% Agarose Gel. Purify the PCR products by cutting the gel piece using sterile scalpel and by using DNA gel extraction kit as per manufacturer's instructions. The purified DNA has to be sent for automated DNA sequencing along with sequencing primer provided in the kit.

The sequence would be analyzed for SNPs in the chromatogram. Mutations are mentioned in the technical specifications.

**PCR product size:** 587 bp



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