MBPCR014 Beta-Thalassemia Detection Kit

Description:

Thalassemia is a group of genetic disorders characterized by quantitative defects in globin chain synthesis with subsequent absence or decrease of hemoglobin production leading to variable degrees of microcytic anemia; it is commonly found in people of Mediterranean, African, Middle Eastern, Indian, Chinese or Southeast Asian origin. Beta-thalassaemia is an autosomal recessive single gene disorder characterized by reduced (β^{+}) or (β^{0}) beta globin chain synthesis leading to reduced hemoglobin A (HbA) synthesis.

Beta-Thalassemia is a disease of considerable public health importance. Apart from causing mortality and morbidity the disease also puts severe strain on family and medical resources. With the advent of DNA diagnostic techniques, it is now possible to offer antenatal diagnosis at 9-11 week of gestation by chorionic villus sampling.

NOTE: Beta-Thalassemia Detection Kit is for *in vitro* use only.

Intended Use:

The kit is designed to detect the 5 different mutations found in Asian population using seven different primers. The details are:

Sr.No.	Description	Fragment size (bp)
1	Internal control (F)	861 (Normal) / 242 (619 deletion)
2	Internal control (R)	
3	Common forward primer (for # 4 to 7)	-
4	Co 8/9 (+G)	214
5	Co 41/12 (-CTT)	443
6	IVS-1nt 5 (G-C)	285
7	IVS-1nt 1 (G-T)	281

This diagnostic kit assures very high sensitivity of detection in clinical samples. The kit is designed for *in vitro* diagnostics and provides qualitative detection.

Principle:

HiMedia's Beta-Thalassemia Detection Kit is a qualitative conventional PCR kit which contains 7 different primers. The presence of amplification product indicates mutation. Conversely, absence of amplified product of expected size indicates absence of mutation. Therefore, in normal condition, no amplification is seen. To overcome the possibility that the absence of product could be due to failure of PCR, an internal control is also included in the kit.

Registered Office :
23, Vadhani Industrial Estate,LBS Marg,
Mumbai - 400 086, India.
Tel. : (022) 4017 9797 / 2500 1607
Fax : (022) 2500 2286

Commercial Office
A-516, Swastik Disha Business Park,
Via Vadhani Indl. Est., LBS Marg,
Mumbai - 400 086, India
Tel: 00-91-22-6147 1919
Fax: 6147 1920, 2500 5764
Email : info@himedialabs.com
Web : www.himedialabs.com

The amplification with primers #1 and #3 will generate a 861 bp fragment which serves as internal control. This primer pair also identifies 619bp deletion, wherein a product of 242 bp instead of 861 bp is formed.

Features:

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Unit Definition:

1u is defined as amount of enzyme that is required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

Storage and Shelf-life:

The provided kit has a shelf-life of 6 months when stored at -20°C. Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce sensitivity of the assay. The kit provided is **stable for 6 months** when stored at mentioned conditions. HiMedia does not recommend using the kit after the expiry date stated on pack.

Kit Contents:

The provided PCR contains:

Components	Reagents provided for 10R (reactions)	Reagents provided for 25R (reactions)	Reagents provided for 50R (reactions)
2X PCR Master Mix	260 µl	650 µl	1.5 ml
Primer Mix (Internal control)	25 µl	60 µl	120 µl
Primer Mix	60 µl	130 µl	260 µl
Nuclease free water **(ML065)**	1 ml	2 ml	4 ml
6X Loading Dye **(ML015)**	100 µl	200 µl	400 µl
100 bp DNA Ladder **(MBT049)**	40 µl	90 µl	180 µl

Sample Material Preparation:

DNA was purified from 200 µl anticoagulated whole blood using HiPurA™ Blood Genomic DNA Miniprep Purification Kit (MB504). The purified DNA is free of any inhibitors and can be used directly for PCR.

General Preparation Instructions:

- Before use, suitable amount of all PCR components should be completely thawed on ice (4°C)
- Perform the amplification reactions in a clean area

- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates
- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area
- Centrifuge the components briefly once thawed

A) Protocol:

Preparation of PCR Reaction Mixture

Add 25 µl of 2X PCR Master Mix **(MBT061)** in a PCR tube

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In the same tube, add 2 µl of internal control primer mix +5 µl thal primer mix (10 pmoles concentration provided)

⇩

Add 1-2 µl of **template DNA** (upto 50 ng of extracted DNA)

⇩

Add nuclease free water **(ML065)** to make the final volume to 50 µl

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Centrifuge the tube briefly at 6000 rpm for about 10 seconds.

⇩

Place the tubes in the PCR machine and set the recommended PCR program (mentioned below)

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Interpret the data using Agarose gel electrophoresis

B. Recommended PCR program:

1. Initial denaturation: 94°C for 5 minutes

2. Cycling Parameters (No. of cycles: 30)
   Denaturation: 94°C for 1 minute
   Annealing: 66°C for 1 minutes
   Extension: 72°C for 1.5 minutes

3. Final Extension: 72°C for 10 minutes

C. After amplification the products may be kept at 4°C overnight or frozen at –20°C for long-term storage.

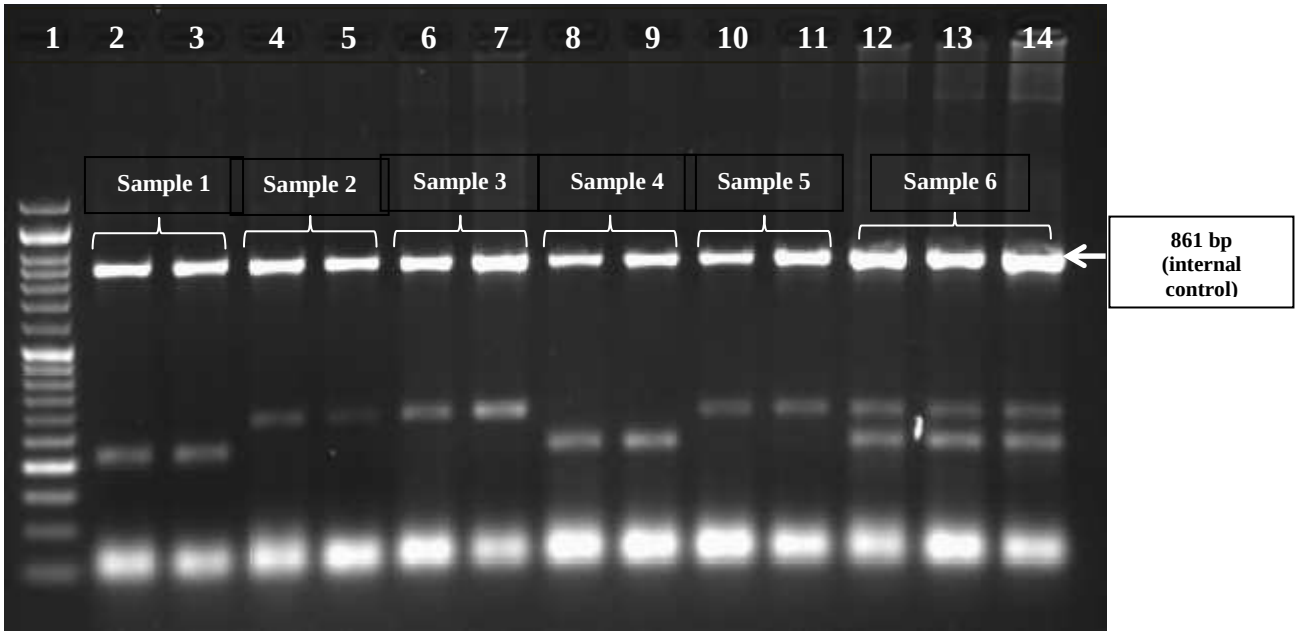
D. Beta-Thalassemia PCR Assay Results Interpretation

- For analysis of the PCR data, load 10 µl of amplicon on a 1.5% Agarose gel along with 1 µl of 6X DNA loading dye (ML015).
- Load 3 µl of 100 bp DNA ladder (MBT049) in separate well.

E. EtBr-staining staining to check results

- Incorporate EtBr in the agarose gel or stain the agarose gel with EtBr for 10-15 min
- Confirm the expected amplicon size comparing with 100 bp DNA marker

Gel image representing amplification of 7 primers indicating mutations from 6 different clinical samples



Lane No.	Sample No.	Interpretation
1	50 bp DNA Ladder	-
2	Clinical Sample No. 1	861 and 214 bp fragment indicating Co 8/9 (+G) mutation
3		
4	Clinical Sample No. 2	861 and 281 bp fragment corresponding to IVS-1 nt 1 (G-T) mutation
5		
6	Clinical Sample No. 3	861 and 285 bp fragment corresponding to IVS-1 nt 5 (G-C) mutation
7		
8	Clinical Sample No. 4	861 and 214 bp fragment indicating Co 8/9 (+G) mutation
9		
10	Clinical Sample No. 5	861 and 281 bp fragment corresponding to IVS-1 nt 1 (G-T) mutation
11		
12	Clinical Sample No. 6	861 and 214 bp fragment indicating Co 8/9 (+G) mutation **&** 285 bp fragment corresponding to IVS-1 nt 5 (G-C) mutation

Specifications:

Sensitivity: Detectable upto 10-100 cfu / ml (mg)

Quality Control:

Each lot of HiMedia's Beta-Thalassemia Detection Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities. Functionally tested in DNA amplification.

Troubleshooting Guide:

Sr.No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of DNA using agarose gel electrophoresis.
			2. Use freshly prepared DNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.
		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	1. Replace all critical solutions 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

The Beta-Thalassemia Detection Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

Product Use Limitation & Warranty

HiMedia guarantees the performance of product in the manner described in the product literature. Beta-Thalassemia Detection Kit is designed and sold for research and *in vitro* purposes only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.
All due care and attention should be exercised in the handling of the products. We recommend all users of HiMedia products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.