

## Instructions for Use

### **MBPCR266    Hi-PCR<sup>®</sup> Human DNA Quantification Kit**

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## 1. Description

Quantification of Human DNA isolated from a forensic evidence sample is an important step for DNA normalization prior to short tandem repeat (STR) DNA analysis. The Hi-PCR® Human DNA Quantification Kit was developed to assess total human DNA, human male DNA, DNA degradation, and/or PCR inhibitors in forensic samples. The assay utilizes two human DNA targets, one to quantify human DNA and the other to assess the integrity (degradation index) of the genomic DNA. In addition to these two targets, a human male target to quantify human male DNA and an exogenous synthetic DNA template as an internal reaction control (IRC) for the assessment of PCR inhibition are included in the kit. These four targets provide a forensic DNA analyst meaningful information about the quantity and quality of the isolated human genomic DNA and the presence of PCR inhibitors in the sample that can be used to determine the most effective workflow and assist downstream interpretation.

**NOTE:** HiMedia's Hi-PCR® Human DNA Quantification Kit is for *in-vitro* use only.

## 2. Intended Use

Recommended to quantify the total amount of amplifiable human DNA in a sample.

## 3. Principle

Real-time polymerase chain reaction, also called quantitative Polymerase Chain Reaction (qPCR) or kinetic Polymerase Chain Reaction, is a laboratory technique based on the principle of PCR. This technique is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end and a quencher dye to the 3' end. Hi-PCR® Human DNA Quantification Kit is a four-dye-target hydrolysis probe-based multiplex assay to quantify the total human and human male DNA in addition to assessing the DNA integrity (degradation index). The kit also includes an internal reaction control (IRC) to detect inhibitors in an amplification reaction.

## 4. Controls

### ➤ Standard Control (S)

Human DNA Quantification (HDQ) Male Genomic Standard is provided in the kit at a concentration of 50 ng/μL. A four-point standard curve for the three targets is generated by preparing a serial dilution of this standard DNA. The standard curves are used to determine the DNA concentration for each target of the unknown sample. **It is ideal to run the standards in duplicates.**

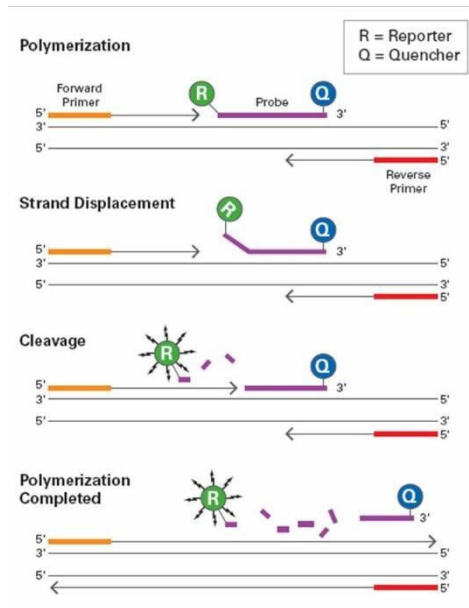
### ➤ Negative Control (N)

Negative control is needed to ensure that the reagents, equipment, and environment used in the assay are not contaminated. In this reaction, Nuclease-free water is used as the template. It is recommended to have a minimum of one reaction of negative control per run.

### ➤ Internal Reaction Control (IRC)

The Internal Reaction Control (IRC) is an exogenous DNA template that should amplify in all samples. The function of the IRC serves for the assessment of PCR inhibition or detecting the failure of amplification in cases where the target sequence is not amplified.

## Diagrammatic representation of preferential binding of probe specific to DNA fragments in Real-time PCR



**Polymerization:** A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' end of the probe respectively

**Strand displacement:** When the probe is intact, the report dye emission is quenched.

**Cleavage:** During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe

**Polymerization completed:** Once separated from the quencher, the reporter dye emits its characteristic fluorescence

While the probe is intact, the proximity of the quencher dye greatly reduces the fluorescence emitted by the reporter dye by fluorescence resonance energy transfer (FRET). The probes are designed such that they anneal within a DNA region amplified by a specific set of primers. During PCR amplification, these probes will hybridize to the target sequences located in the amplicon i.e., the DNA. As the *Taq* DNA polymerase replicates the template with the bound probe, the 5'-nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The result of the cleavage of the probe is the separation of the reporter dye from the quencher dye and increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. Hence, fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. Thus, the inclusion of the probe does not inhibit the overall PCR process.

### 5. Features

- Fast and robust.
- Highly sensitive and specific.
- Includes all reagents and controls.

### 6. Types of Specimens

- DNA samples extracted from different forensic casework samples.
- Before DNA extraction, specimens are to be preprocessed.
- After extraction, store the extracted DNA samples at  $-20^{\circ}\text{C}$  for short period storage and  $-70^{\circ}\text{C}$  or lower for long period storage.

### 7. Specimen collection and Handling

- Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding.
- Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with other body fluids.
- Safety guidelines may be referred to in individual safety data sheets.

### 8. Storage and Shelf life

- The provided kit has a shelf-life of 12 months when stored between  $-10^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$ .
- Repeated thawing and freezing of PCR reagents should be avoided, as this may reduce the sensitivity.

- If the reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw.
- This kit can be used for a maximum of 6 repeats of freezing and thawing.
- Degradation of sample DNA specimens can also reduce the sensitivity of the assay.
- HiMedia Laboratories does not recommend using the kit after the expiry date stated on the pack.

## 9. Kit Contents

Components	Product code	Reagents provided for	
		50R* (µL)	100R* (µL)
HDQ Mastermix	DS1750	270	530
HDQ Primer-Probe Mix	DS1300	486	954
Internal Reaction Control (IRC)	DS1301	54	106
HDQ Male Genomic Standard (50 ng/µL)	DS1302	20	40
HDQ Dilution Buffer	DS1303	500	1000

\* For a 20 µL PCR reaction

## 10. Devices, Materials, and Reagents required but not provided

All materials are available through [www.himedialabs.com](http://www.himedialabs.com)

Product name	Product Code
<b>Real-Time PCR Instrument and equipment</b>	
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
TabSpin™ Microcentrifuge	LA1089/LA1090
<b>Automated nucleic acid extraction system and materials</b>	
InstaNx® Mag32	LA1096
HiPurA Multi Sample Pre-filled Plates for Insta NX Mag32	MB554MPF32200
<b>Kits and Reagents</b>	
HiPurA® Forensic Multi Sample DNA Purification Kit	MB580
<b>Tubes, plates and other consumables</b>	
Varivol II Micropipette-10 (Capacity: 0.5 to 10 µL)	LA611
Varivol II Micropipette-100 (Capacity: 10 to 100 µL)	LA615
Varivol II Micropipette-1000 (Capacity: 200 to 1000 µL)	LA614
Barrier Tips, Maximum capacity 10 µL	LA749/LA749A
Barrier Tips, Maximum capacity 200 µL	LA751/LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859/LA859A
0.2 mL 8-strip tubes & optically clear flat caps for PCR	PR17
0.2 mL PCR tubes	PW1255
0.2 mL PCR Plates	PR3/PR19
Optical Sealing film	PR18

## 11. Warning and Precautions

Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred to in the safety data sheets of the product.

## 12. Limitations

Although rare, mutations within the highly conserved regions of the target genes covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the target

regions in these cases. The validity and performance of the assay design are revised at regular intervals.

### 13. General Preparation Instructions

- Before use, all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area, preferably in a biosafety cabinet.
- The use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous DNA templates.
- Extract and store the positive control sample (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.

### 14. Kit Compatibility with Real-Time PCR systems:

Hi-PCR® Human DNA Quantification Kit contains fluorophores compatible with

- Insta Q96® - 6.0 Real-Time PCR System (HiMedia Laboratories Pvt. Ltd.)
- QuantStudio™ 5 Real-Time PCR Instrument (Thermo Fisher)

**Note: Ensure that the Real-Time PCR system is calibrated for FAM, JOE, TexasRed, and Cy5 dye and is maintained according to the manufacturer’s instructions and recommendations.**

### 15. Preparation of DNA standards

- Equilibrate HDQ Male Genomic Standard (50 ng/μL) (DS1302) to room temperature for at least 15 minutes.
- Using HDQ Dilution Buffer (DS1303), prepare 10-fold serial dilutions of the HDQ Male Genomic Standard (50 ng/μL) (DS1302) for quantitation of Human, Male and Degradation targets in the unknown DNA samples.
- Kindly prepare the dilutions in the following manner:
  - After the initial thaw, store the HDQ Male Genomic Standard (50 ng/μL) (DS1302) and HDQ Dilution Buffer (DS1303) at 4°C till the dilutions are prepared.
  - Label four tubes with the following concentrations: 5 ng/μL, 0.5 ng/μL, 0.05 ng/μL and 0.005 ng/μL to prepare a four-point standard curve.
  - Prepare fresh serial dilutions of the HDQ Male Genomic Standard (50 ng/μL) (DS1302) as indicated in Table 1.
  - Give a pulse vortex and centrifuge for 5 seconds at 3000 rpm the HDQ Male Genomic Standard (50 ng/μL) (DS1302) and each dilution to collect all liquid at the bottom of the tube before use. Change pipette tips between dilutions.

**Table 1. Serial Dilution of the HDQ Male Genomic Standard (50 ng/μL) (DS1302) (10-fold dilutions)**

Final DNA Concentration (ng/μL)	Original DNA Concentration (ng/μL)	Volume of HDQ Male Genomic Standard	Volume of HDQ Dilution Buffer
5	50	5	45
0.5	5	5	45
0.05	0.5	5	45
0.005	0.05	5	45

Note: Multiple cycles of freeze-thaw are not recommended for the HDQ Male Genomic Standard (50 ng/μL) (DS1302). Longer-term storage is not recommended. Be sure to change pipette tips between dilutions. Change gloves after handling high-concentration DNA.

### 16. Protocol for PCR Master Mix Preparation

- a. In the “Master mix Preparation” area, thaw all components from the kit on ice, mix by inverting the tubes, and centrifuge the reagents for several seconds. Keep on ice for later use.

- b. Based on the number of specimens to be tested (N), including the standards and NC, calculate the volume of the components to be added as N\* volume of 1X.
- c. Kindly add the components mentioned in the following order

Components	Product code	Volume to be added for 1R (for a 20 µL reaction)
HDQ Mastermix	DS1750	5 µL
HDQ Primer-Probe Mix	DS1300	9 µL
Internal Reaction Control (IRC)	DS1301	1 µL
Template DNA / Standards / Negative Control	-	5 µL
Total volume	-	20 µL

- d. Use 1.5 mL nuclease-free centrifuge tube(s) for the preparation of the reaction system. After all the reagents are added, mix them thoroughly and centrifuge for several seconds.
- e. Load 15 µL of the master mixture into the PCR reaction strips/plate.
- f. Add 5 µL of template DNA / diluted standards / Negative Control.
- g. For negative control, use HDQ Dilution Buffer
- h. Tightly cap the strips or seal the plate using an optically clear adhesive film. Briefly, spin the strips/tubes to settle the reagent to the bottom of the tube. Place the plate/strips in a Real-time PCR machine and set the PCR program.

## 17. Preparing a Run Template

### a. InstaQ 96® 6.0 Real-Time PCR System

- i. Turn on the computer associated with the InstaQ 96® 6.0 Real-Time PCR System.
- ii. Turn on the InstaQ 96® 6.0 Real-Time PCR System.
- iii. Open the InstaQ 96® (six) software.
- iv. The template file with the dyes used, target names, DNA concentrations, plate setup and program settings are provided. Alternatively, the template file is available from HiGenoMB® Forensic-ID DNA Quantification Software (MBES01).
- v. Open the template file:
  - a. In the Detector tab, modify the name of the Experiment Name.
  - b. Dyes used: The following mentioned dyes are used for the respective targets

Target Name	Dye	Channel
HDQ_Male	FAM	1
HDQ_Human	JOE	2
HDQ_Degradation	TexRed	3
HDQ_IRC	CY5	4

- c. In the Sample tab, the standards with the DNA concentrations are predefined.
  - i. Add sample information > Enter ID in Sample ID > Enter.
  - ii. Delete sample information > Select the sample > Delete. To delete all samples, click **Clear All**.
  - iii. Import/Export sample information:
    - 1. To Export: Export Samples Info. The file will be exported in CSV file format.
    - 2. Enter the required sample information.
    - 3. To Import: Import Samples Info > Select the sample information file in CSV format.
- d. In the Plate tab, mark detectors and samples exactly like the reaction tubes placed inside the block.
  - i. Select the well and tick the corresponding target/reporter dye added.
  - ii. Select the sample to be added from the list.

- e. In the Program tab, the cycling conditions are predefined and not be modified.
- f. Running the experiment
  - i. Select “Save As” to name and save the experiment.
  - ii. Load plate and select “Start Run”.

**b. QuantStudio™ 5 Real-Time PCR System**

- i. Turn on the computer associated with the QuantStudio™ 5 Real-Time PCR System
- ii. Turn on the QuantStudio™ 5 Real-Time PCR System.
- iii. Open the QuantStudio™ Design and Analysis Software.
- iv. The template file with the dyes used, target names, DNA concentrations, plate setup and program settings are provided. Alternatively, the template file is available from HiGenoMB® Forensic-ID DNA Quantification Software (MBES01).
- v. Open the template file.
  - a. In the Experiment Properties window, modify the name. The rest of the fields need not be modified as they are predefined in the template file.
  - b. Select “Next”.
  - c. In the Method window, the cycling conditions are predefined and not be modified.
  - d. Select “Next”.
  - e. In the Plate window, in the Assign Targets and Samples, click on Advanced Setup tab.
    - i. In the Targets section of the Advanced Setup tab, the targets are predefined and not to be modified.

Target Name	Reporter	Quencher
HDQ_Male	FAM	None
HDQ_Human	VIC	None
HDQ_Degradation	ROX	None
HDQ_IRC	CY5	None

- iii. In the Samples section of the Advanced Setup tab, the standards with the DNA concentrations are predefined and not to be modified.
- iv. Click on Add > Enter ID in Sample ID > Enter.
- v. Delete Sample by clicking on cross button.
- g. Mark detectors and samples exactly like the reaction tubes placed inside the block.
  - i. Select the well and tick the corresponding target/reporter dye added.
  - ii. Select the sample to be added from the list.

**18. Recommended PCR program**

**a. InstaQ 96® 6.0 Real-Time PCR System**

- |                         |   |                              |   |                   |
|-------------------------|---|------------------------------|---|-------------------|
| 1. UNG Incubation       | : | 25°C for 02 minutes          | } | No. of cycles: 45 |
| 2. Initial denaturation | : | 95°C for 02 minutes          |   |                   |
| 3. Denaturation         | : | 95°C for 10 seconds          |   |                   |
| 4. Annealing            | : | 55°C for 1 minute (Sampling) |   |                   |
| Sampling                | : | FAM, JOE, TexasRed, and Cy5  |   |                   |

**b. QuantStudio™ 5 Real-Time PCR System**

- |                          |  |                     |
|--------------------------|--|---------------------|
| 1. UNG Incubation        | : 25°C for 02 minutes                                      | } No. of cycles: 45 |
| 2. Initial denaturation  | : 95°C for 02 minutes                                      |                     |
| 3. Denaturation          | : 95°C for 10 seconds                                      |                     |
| 4. Annealing<br>Sampling | : 55°C for 1 minute (Sampling)<br>: FAM, VIC, ROX, and Cy5 |                     |

**19. Data analysis**

The following conditions should be met for a valid test:

Control	Detection channel			
	Male	Human	Degradation	IRC
Standards	+	+	+	+/-
Negative Template Control	-	-	-	+

The Hi-PCR® Human DNA Quantification Kit assay is highly sensitive. Spurious signals may be obtained which are possibly the result of ambient DNA or sporadic signal from the Male, Degradation and Human targets.

Ct value	Result
≤ 40	Detected (+)
> 40 or N/A	Not detected (-)

**IMPORTANT! Before using the highly sensitive Hi-PCR® Human DNA Quantification Kit, assess the cleanliness of your environment. Use stringent laboratory cleanliness protocols to minimize contamination.**

**20. Data Interpretation**

The following parameters should be evaluated for each run.

Target	InstaQ 96® 6.0 Real-Time PCR System		QuantStudio™ 5 Real-Time PCR System	
	Ct threshold	Baseline	Ct threshold	Baseline
HDQ_Male	Auto	Auto	Auto	Auto
HDQ_Human	Auto	Auto	Auto	Auto
HDQ_Degradation	Auto	Auto	Auto	Auto
HDQ_IRC	Auto	Auto	Auto	Auto

View the Ct values for the quantification standard reactions and the Slope values, Correlation / R<sup>2</sup> and Efficiency.

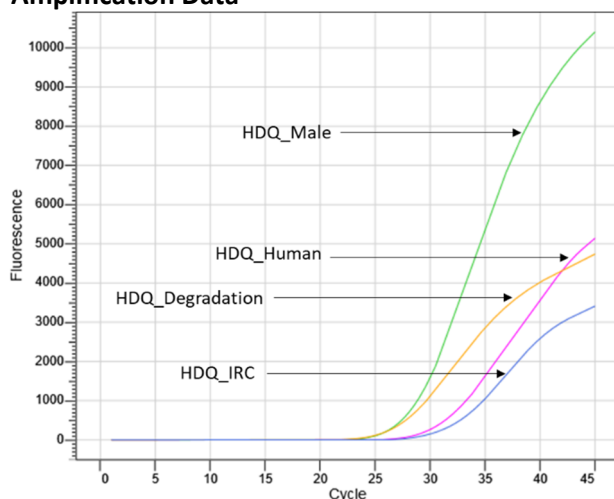
Target	Slope values	R <sup>2</sup>	Efficiency
HDQ_Male	-3.1 to -3.6	≥0.990	> 90% and <110%
HDQ_Human	-3.1 to -3.6	≥0.990	> 90% and <110%
HDQ_Degradation	-3.1 to -3.6	≥0.990	> 90% and <110%

The IRC is expected to have Ct values no more than 2 units above the mean IRC Ct for all quantification standards on the plate. Ct values higher than these for the IRC is indicative of a PCR inhibitor present in the reaction, including an excessively high concentration of DNA. The IRC in no-template samples must have a Ct between 32.1 and 34.1 cycles.

## 21. HID calculation

To obtain the male:female ratio, degradation index, sample inhibition and dilutions for downstream applications, kindly use the HiGenoMB® Forensic-ID DNA Quantification Software (MBES01). Export the results in the excel format. Import the excel file in the HiGenoMB® Forensic-ID DNA Quantification Software (MBES01) for calculation and dilutions.

## 22. Amplification Data



Sr. No.	Target	C <sub>t</sub> value
1	HDQ_Male	30.33
2	HDQ_Human	32.72
3	HDQ_Degradation	28.53
4	HDQ_IRC	32.9

Note: Image representing amplification plot of Human DNA Detection with Ct values using HiMedia's Hi-PCR® Human DNA Quantification Kit. (Ct values provided in table are for representation)

## 23. Performance Evaluation

### ➤ Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the HiMedia's Hi-PCR® Human DNA Quantification Kit was conducted using standard human DNA. The detectable limit of the HiMedia's Hi-PCR® Human DNA Quantification Kit was determined to be  $\leq 1$  pg/ $\mu$ L.

### ➤ Inclusivity - Analytical Sensitivity

*In-silico* analysis for the assessment of inclusivity for the HiMedia's Hi-PCR® Human DNA Quantification Kit was conducted by mapping the primers and probes against all the available sequences in GenBank. The HiMedia's Hi-PCR® Human DNA Quantification Kit targets 100% of the known hits.

### ➤ Cross-reactivity - Analytical Specificity

Wet testing analysis was performed against the following pathogens. No cross-reaction was observed with any strains.

<i>Escherichia coli</i>	<i>Saccharomyces cerevisiae</i>	Rabbit	Bovine Female
<i>Klebsiella pneumoniae</i>	<i>Candida albicans</i>	Pig	Bovine Male
<i>Pseudomonas aeruginosa</i>	Dog	Chicken	Human Male
<i>Staphylococcus aureus</i>	Cat	Sheep	Human Female
<i>Streptococcus pneumoniae</i>	Horse	Mouse	
<i>Enterococcus faecalis</i>	Rat	Goat	

## 24. Evaluation

Each lot of HiMedia's Hi-PCR® Human DNA Quantification Kit is tested against predetermined specifications to ensure consistent product quality.

## 25. Quality Control

Each lot of HiMedia's Hi-PCR® Human DNA Quantification Kit is assayed for contaminating endonuclease, exonuclease and non-specific DNase activities and has been functionally tested in amplification.

## 26. Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	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 a large volume master mix, vortex thoroughly and aliquot into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a Real-Time PCR instrument.
		Pipetting error	C <sub>t</sub> values of replicates can show increased variation due to poor laboratory technique 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.
4.	No signal with positive controls	Incorrect programming of the temperature profile of the thermal cycler	Compare the temperature profile to the manual.

## 27. Safety Information

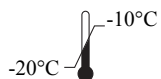
HiMedia's Hi-PCR® Human DNA Quantification Kit is for laboratory use only, not for drug, household, or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

## 28. Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures while disposing the infectious materials. All used material must be decontaminated and disposed of in accordance with current laboratory techniques.

## 29. Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For technical assistance, please mail to [mb@himedialabs.com](mailto:mb@himedialabs.com).



Storage temperature



Do not use if package is damaged



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