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Product Information

Instructions for Use

MBPCR277 Hi-PCR® Human Y DNA Quantification Kit

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MBPCR277 **Hi-PCR® Human Y DNA Quantification Kit**

Table of Contents

1. Description

Many labs are interested in precisely determining the sex of humans or fetuses using molecular biology techniques. Numerous quick and precise techniques for "sexing" human DNA have been created, with an emphasis on finding Y-specific chromosomal sequences. The Hi-PCR® Human Y DNA Quantification Kit was developed to detect and quantify human Y DNA in different types of samples. The assay utilizes 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 two targets provide a DNA analyst meaningful information about the quantity and quality of the isolated human Y 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 Y DNA Quantification Kit is for *in-vitro* use only.

2. Intended Use

Recommended to quantify the total amount of amplifiable human Y 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 Y DNA Quantification Kit is a two-dye-target hydrolysis probe-based multiplex assay to quantify human male DNA. 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.**

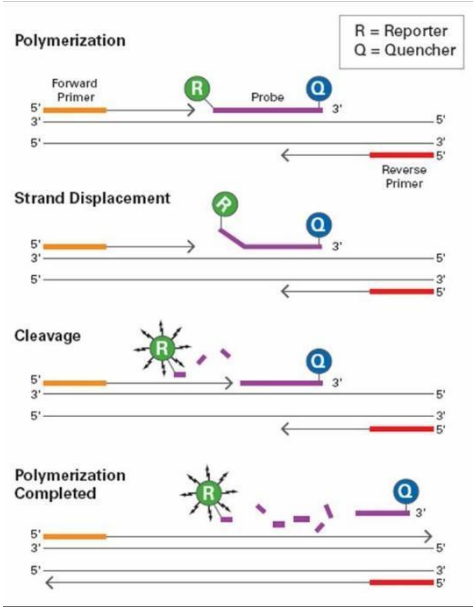
- **Negative Control (N)**

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°C for short period storage and -70˚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°C to -20°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		
		25R* (µL)	50R* (µL)	100R* (µL)
Y DNA Mastermix	DS1749	135	270	530
Y Primer-Probe Mix	DS1747	243	486	954
Internal Reaction Control (IRC)	DS1301	27	54	106
HDQ Male Genomic Standard (50 ng/µL)	DS1302	10	20	40
HDQ Dilution Buffer	DS1303	250	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

Product name	Product Code
Real-Time PCR Instrument and equipment	
Insta Q96®AG Real time PCR System, 96 well block, 5 channels	MBLA027
Insta Q96®AG 6.0 Real time PCR System, 96 well block, 6 channels	MBLA028
Insta Q96® Plus Real time PCR System, 96 well block, 5 channels	LA1073
Insta Q96® - 6.0 Real time PCR System, 96 well block, 6 channels	LA1074
Insta Q96® Real time PCR System, 96 well block, 5 channels	LA1012
Insta Q48® M4 Real time PCR System, 96 well block, 4 channels	LA1023
Insta Q48® M2 Real time PCR System, 96 well block, 2 channels	LA1024
TabSpin™ Microcentrifuge	LA1089/LA1090
HiPer® Mini Plate Centrifuge	LA1099
Automated nucleic acid extraction system and materials	
Insta NX® Mag16, Insta NX® Mag16^{Plus}	LA1118, MBLA018
Insta NX® Mag32, Insta NX® Mag32^{Plus}	LA1096, MBLA019
Extraction Kits	
HiPurA® Pre- Filled cartridges for DNA Extraction	MB554PC16200
HiPurA® Multi-sample Pre-filled Plates for DNA Extraction	MB554MPF16200
HiPurA® Multi sample Pre-Filled Plates for Insta NX® Mag32	MB554MPF32200
Tubes, plates, and other consumables	
Varivol II Micropipettes (Capacity: 0.5 to 10 µL/10 to 100 µL/200 to 1000 µL)	LA611/LA614/LA615
µPet Autoclavable Micropipettes (Capacity: 0.5 - 10 µL/10 - 100 µL/20 - 200 µL/100 - 1000 µL)	LA955/LA958/LA959/LA960
Q4Pet Autoclavable Micropipette (Capacity: 0.5 to 10 µL/10 to 100 µL/100 - 1000 µL)	MBLA009/MBLA011/MBLA008
Barrier Tips, Maximum capacity 10 µL	LA749A
Barrier Tips,100µl Max capacity 100 µL	LA1104A
Barrier Tips, Maximum capacity 200 µL	LA751A
Barrier Tips, Maximum capacity 1000 µL	LA859A

8-strip tubes & optically clear flat caps for PCR	PR17, PR22, PR23
PCR Tubes, 0.1 mL, 0.2 mL; PCR Plates	PW1255/PR2/PR3/PR19
Optical Sealing film	PR18
1.5 ml nuclease free Micro centrifuge tubes	PW146

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:

Ensure that the Real-Time PCR system is calibrated for FAM, and Cy5 dye and is maintained according to the manufactureŕ 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)
Y DNA Mastermix	DS1749	5 µL
Y Primer-Probe Mix	DS1747	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. Recommended PCR program

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

18. Data analysis

The following conditions should be met for a valid test:

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

The Hi-PCR® Human Y 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 targets.

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

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

19. Data Interpretation

The following parameters should be evaluated for each run.

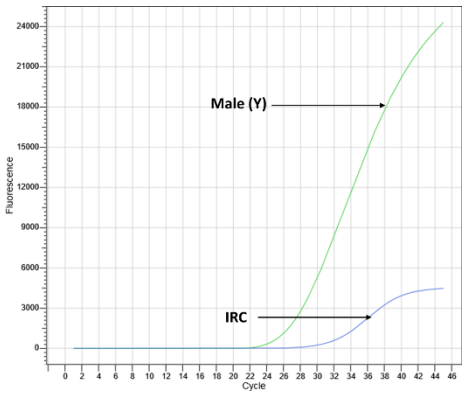
Target	InstaQ 96® Series		QuantStudio™ 5 Real-Time PCR System	
	Ct threshold	Baseline	Ct threshold	Baseline
HDQ_Male	Auto	Auto	Auto	Auto
HDQ_IRC	Auto	Auto	Auto	Auto

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

Target	Slope values	R^2	Efficiency
HDQ_Male	-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.

20. Amplification Data



Sr. No.	Target	C_t value
1	HDQ_Male	27.29
2	HDQ_IRC	32.22

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

21. Performance Evaluation

➢ Limit of Detection (LoD) - Analytical Sensitivity

Sensitivity for the HiMedia's Hi-PCR® Human Y DNA Quantification Kit was conducted using standard human DNA. The detectable limit of the HiMedia's Hi-PCR® Human Y DNA Quantification Kit was determined to be ≤ 1 pg/µL.

➢ Inclusivity - Analytical Sensitivity

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

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

22. Evaluation

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

23. Quality Control

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

24. 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_t values of replicates can show increased variation due to poor laboratory technique or imprecise pipettes.

Please refer disclaimer Overleaf.

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.

25. Safety Information

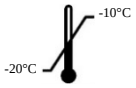
HiMedia's Hi-PCR® Human Y DNA Quantification Kit is for laboratory use only, not for drug, household, or other uses. Take appropriate laboratory safety measures and wear gloves when handling.

26. 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.

27. 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.

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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Disclaimer :

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