# Insta Q96®-6.0 LA1074



Real-Time PCR System







## Introduction

With a vision to redefine PCR based solutions, HiMedia Laboratories Pvt. Ltd. launched the Insta-Q Real-Time PCR Platform with its unique range of features making it an exclusive Real-Time PCR System in the market. Our **User-Friendly State of the Art** machine can measure amplification as it occurs, cycle by cycle, thus resulting in precise & accurate quantification.

The Insta Q96® - 6.0, Real-Time PCR System is a fully integrated quantitative PCR amplification, detection and data analysis platform. The latest design combines a thermal cycler, an advanced optical system with individual LED excitation source and an intuitive data analysis software. The **Gradient Feature** aids in easy assay optimization.

These robust machine is genuinely **Open Systems** – which enable the user to decide the choice of reagents and kits to be run. The machine come with **Factory Calibrated Filters**. Re-calibration is required only in case of major machine upgradations. A unique feature of the Insta Q96® - 6.0 is the qPCR optics available in a more flexible format. To get accurate results, the **Robotic Arm Scans Individual Well** which **Eliminates** the use of Passive Reference Dye - **ROX dye**.

The software is equipped to export the raw data in multiple formats such as **Excel, Images, Text, Pdf** thus allowing results to be viewed in common programs.

The sensitivity and specificity of the Insta-Q Series of instruments is impressive and will help the user to generate **Faster**, **Hassle-free and Reliable results** to achieve desired research goals.

#### **Features**

- Truly Open System [Compatible with Kits and Reagents of other companies].
- ROX independent Real-Time PCR system.
   Normalization with ROX dye not required.
- Customizable dye library to create new excitation/detection wavelength combinations in given range and hence future proof.
- User Friendly Software facilitates simple assay set up and data interpretation for Plus/minus assay utilizing Internal Positive Controls, comparative data analysis(Ct value), Standard curve, Relative Standard Curve, Gene expression analysis, Allelic Discriminations, Dye discrimination multicomponent algorithm.
- Auto Gain intensity function for fluorescence adjustments.
- 12 different gradient temperatures (1°C to 36°C gradient range).
- Wireless connectivity.
- Factory calibrated filters, no need of calibration after new dye added or in future.

Dye Library	
Channel Wavelength	Dyes
470nm – 525nm	FAM, SYBR, EvaGreen®
523nm – 564nm	HEX, JOE, TET, VIC, Cal Fluor® Gold 540 / Yakima Yellow
571nm – 612nm	ROX, TexRed, Cal Fluor® Red 610 / JUN
628nm – 692nm	CY5, Mustang Purple / Quasar 670 / Pulsar 650
678nm – 718nm	Cy5.5, Quasar 705
550nm – 585nm	TAMRA, NED, Cy3, ABY

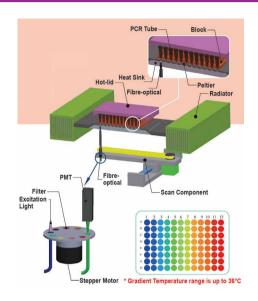
- 1. Innovative 3D Hotlid Design and Technology
- 2. 96 Wells High Throughput
- 3. Gradient facility
- 4. Well to well individual scanning
- 5. Optical fibre based Photo Multiplier Tube Technology for detection





## **Working Principle of the Machine**

- Ferrotec Peltier technology used for thermal cycling during PCR assay.
- LED based excitation source with advanced fibre optic transmission technology for Sensitive and Reliable photoelectric detection system.
- Photo multiplier Tube (PMT) detects fluorescent emission.
- Stepper motor with robotic arm containing optical fibre used for individual well scanning.
- Scanning time period: 5.5 seconds for 96 wells.



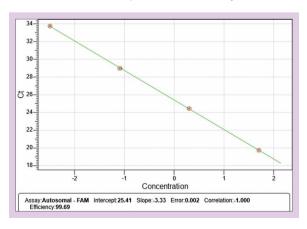
#### Analysis procedures supported by Insta-Q Series software



## **Absolute Quantification**

- Absolute quantification is achieved by comparing the Ct values of the test samples to a standard curve.
- The result of the analysis is quantity of nucleic acid (copy number, unit mass) per given amount of sample (per cell, per ng of total RNA).
- Absolute quantitation uses serially diluted standards of known concentrations to generate a standard curve.
- Standard curve produces a linear relationship between Ct and initial amounts of total DNA or cDNA from RNA of the Gene of interest (GOI), allowing the determination of the concentration of unknowns based on their Ct values.
- The linearity is denoted by the R squared (r²) value (r is Pearson Correlation Coefficient) and should be very close to 1 (> 0.985).
- The efficiency of both the standard curve and sample reactions should be between 90 and 110%.

#### **Standard Quantification Assay**



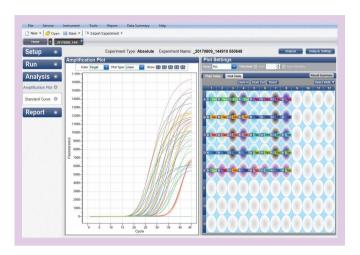




## **Plotting a Standard Curve**

- In absolute quantification, the quantity (e.g., copy number or unit mass) of the unknown sample is interpolated from a range of standards of known quantity.
- To construct a standard curve, a template with known concentration is required.
- Dilution of this template is then performed and these dilutions serve as the standards. The unknown test samples are assayed with the standards in the same experimental run.
- The standard curve constructed from the diluted standard template can then be used to determine the target quantity in the unknown sample by interpolation, similarly to using molecular size standards to determine the molecular size of an unknown DNA band on an agarose gel.
- Standard curve can be imported from previous run experiments. It can be imported only in standard curve assays. Hence standards need not be run every time.

#### **Software Analysis Interface**



## **Relative Quantification**

- Let's get the nomenclature settled.
  - The gene of interest whose expression is getting determined is the target gene.
  - The housekeeping gene whose expression is unregulated is called the reference gene.
  - The sample (or group of samples) being used as a control is the calibrator sample.
  - Finally, the sample (or group) that is being treated or tested for differences is the test sample.
  - The ratio of the target gene expression in the test sample over the calibrator sample is interchangeably called the expression fold change or relative gene expression.
- Amplification efficiency of the reaction is an important consideration when performing relative quantitation.
- Past methods of calculating gene expression have assumed the amplification efficiency of the reaction is ideal, or 1.

- Actual amplification efficiency values for a particular reaction can be established via a standard curve measurement during assay design, and multiple standard curves should be run to verify that this efficiency measurement is reproducible.
- Although absolute quantification can be useful in determining absolute quantities of target, the majority of scientific questions regarding gene expression can be accurately and reproducibly answered by measuring the relative concentration of the GOI in unknown samples.
- Differences in Ct value between an unknown sample and reference sample are expressed as fold-changes (i.e., up- or down- regulated) relative to the reference sample and thereby the results are expressed as a target/reference ratio.

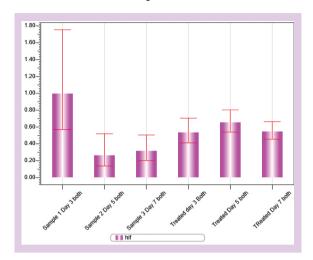




#### **Features**

- Automated calculation of ΔCt and ΔΔCt values by software.
- Exact and final RQ values provided by software at the end of the assay.
- Easy and hassle free transfer of data to Excel or Word format on a Single Click.
- Option to import Standard curves run from other experiments in RQ assays as well.
- Normalization to multiple endogenous controls.

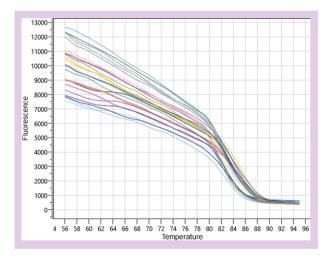
## Relative Quantification



## **High-Resolution Melt Analysis**

- The principle of HRM is the same as a Low-Resolution Melt curve, except that the temperature difference between each fluorescence reading is reduced.
- During a Low-Resolution Melt curve analysis, the temperature increases are typically in 0.5 °C steps, but for HRM this is reduced to 0.008 - 0.2 °C increments.
- This allows a much more detailed analysis of the melting behaviour.
- HRM sensitivity and reliability has been improved with the use of a variety of new dsDNA intercalating dyes viz., -LCGreen (R), SYTO9, EvaGreen (R), Chromofy and BEBO.

#### **HRM** data



## **Features**

- HRM assays can be run using the same software.
   Saves the trouble of learning and procurring a new software.
- No external calibration required for running HRM assays.
- Cost effective compared to other genotyping technologies such as sequencing and TaqMan SNP typing.
- Fast and able to accurately genotype huge numbers of samples in rapid time.
- Fast and high-throughput analysis of post-PCR of genetic mutations or variance in nucleic acid sequences.
- With a good quality, HRM assay powerful genotyping can be performed by non-geneticists in any laboratory with access to an HRM capable Real-Time PCR machine.





### HRM has renewed interest in the utility of DNA melting for a wide range of uses, including:

- ★ Mutation discovery (gene scanning)
- → Species identification
- ★ Screening for loss of heterozygosity
- → Somatic acquired mutation ratios
- → DNA fingerprinting
- → HLA compatibility typing
- ◆ SNP genotyping

- ★ Association (case/control) studies
- → Characterization of haplotype blocks
- → Allelic prevalence in a population
- → DNA methylation analysis
- Identification of candidate predisposition genes
- → DNA mapping

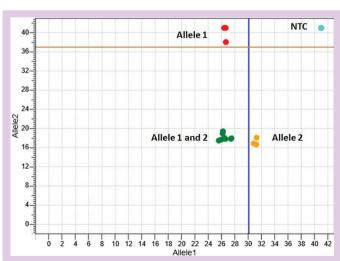
## Single Nucleotide Polymorphism (SNP)

- A Single Nucleotide Polymorphism or SNP is a DNA sequence variation occurring when a single nucleotide in the genome differs between members of a species or two allele of a gene.
- Probe based SNP Genotyping Assays provide a highly flexible technology for detection of polymorphisms within any genome.
- Probe Assays have a simple workflow and provide a quick way to generate genotyping data.

### **Features**

- Auto Call and Manual call options
- Easy and colour coded Scatter plot based on SNP assay analysis

SNP



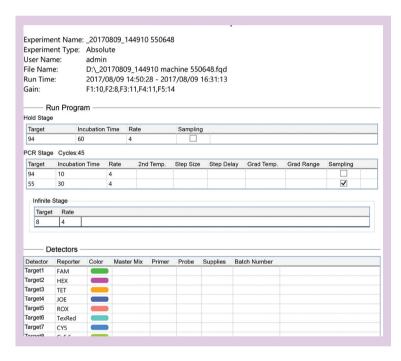


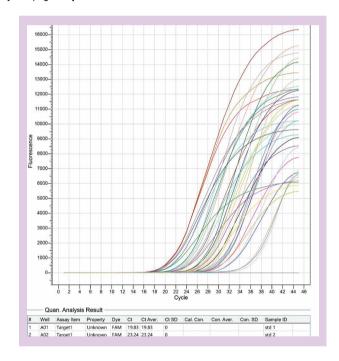


## **Report Generation**

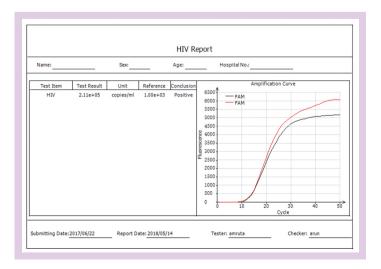
- Generate automatic assay reports at the end of PCR run.
- Customize assay reports as per requirement using built in report editor
- All in one consolidated report for
  - Accurate & concised experimental details
  - Basic experiment information
  - Experiment process
  - Plate diagram
  - Amplification curve
  - Result table with Ct values

#### Consolidated Report / QC Report





#### **Report Template**







## **Product Hardware**

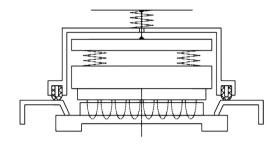
#### **Hot Lid Technology**

- Innovative 3D Hot Lid. It consists of a Pressure Box which exerts uniform pressure on the plate module through 6 compression springs.
- Obtain perfect sealing and avoid sample evaporation or overloading due to lid imbalance.
- The aluminium plate fits snugly on the PCR plate with a certain amount of pressure. This airtight seal prevents the cold air and hot air connection on the module. Thus, the module bears dynamic temperature uniformity.

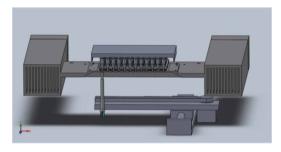


Bottom scanning technology for well-to-well individual scanning.

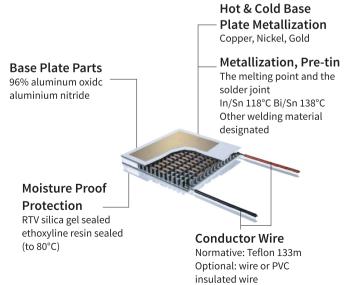
- Precise optical path system combined with sensitive PMT system detects fluorescence activity accurate, sensitive & reliable.
- The probe has a long-life LED light source which requires no maintenance.







- The new model of Thermo Electric (TE) base plate (72 series) has a longer life span.
- The new adhesive technology used with the advanced semi-conductor substrate:
  - i. Improves the performance of the TE base plate under highly humid conditions.
  - ii. Greatly improves the life span of the TE base plate

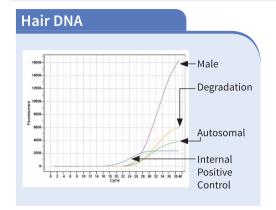


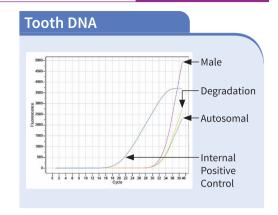


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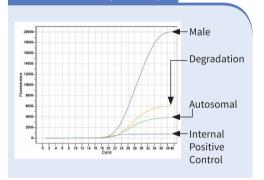


## **Graphical Representation of Data Obtained Using LA1074**



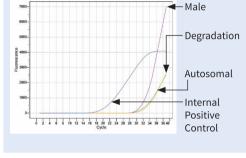


#### Muscle tissue (foetus)

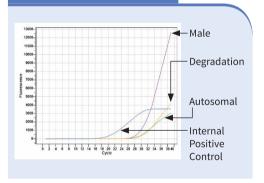




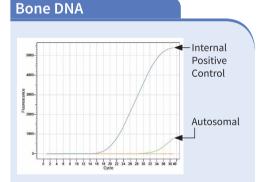
**Bone DNA** 



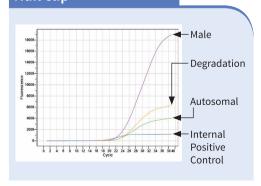
#### **Blood-stained fabric**

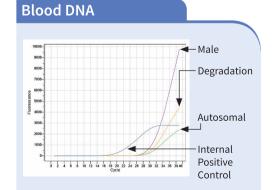






#### Nail clip





Disclaimer: The images/graphs presented here are for representation purposes only and are obtained by using a standard human DNA quantification kit.





## HiGenoMB® Forensic-ID DNA Quantification Software (MBES01)

HiMedia's HiGenoMB® Forensic-ID DNA Quantification Software is a user friendly, Web-Based application developed specifically to assist forensic experts who work on Human Identification (HID). HiMedia's HiGenoMB® Forensic-ID DNA Quantification Software is Novel and First open software developed and designed for use with Insta-Q96® 6.0 (LA1074). The software supports all commercially available Human DNA Quantification Kits.

#### **Features**

- The HiMedia's HiGenoMB® Forensic-ID DNA Quantification Software is designed to perform Real-Time analysis without any data alteration.
- Based on the kit processed in Insta-Q96® 6.0 (LA1074), our software automatically gives information about the Quantity of DNA and has additional built-in features to calculate the Degradation Index (sample quality assessment), Human Male:Female Mixtures or Ratio, Sample Inhibition with IPC Shift and other parameters.
- The HiMedia's HiGenoMB® Forensic-ID DNA Quantification Software also helps to prepare a dilution series to setup various downstream STR experiments.
- The final values are presented using Dynamic Report Generation Algorithm in non-editable reporting format (.pdf) with IP based Time Stamping.
- The software has Secure User Access, which is encrypted using advance cryptography with Auto Logout Facility.



## HiGenoMB® Primer and Probe Design Software (MBES02)

#### **Features**

- Design primers or assays for PCR, qPCR, or sequencing (any species).
- Design algorithm includes multiple checks to reduce primer-dimer formation.
- Provides flexible sequence entry and batch entries.
- Licensed Software

HiGenoMB® Forensic-ID DNA Quantification Software	MBES01-1LC	1lc	On request
HiGenoMB® Primer and Probe Design Software	MBES02-1LC	1lc	On request







# Human Identification

Human DNA Quantification Kits are required in forensic laboratories for simultaneous quantitative and qualitative assessment of total human and human male DNA in a single, highly sensitive real-time PCR reaction. HiMedia's Insta-Q96® series validated for the latest forensic human & male DNA Quantification Kits helps to deliver accurate, sensitive and specific results that work together with the HiGenoMB® Forensic-ID DNA Quantification Software to enable informative and integrated workflow decisions.

#### **COMPATIBILITY CHART**

Parameters	Quantifiler™ Human DNA Quantification Kit *	Quantifiler™ Y Human DNA Quantification Kit *	Quantifiler™ Duo DNA Quantification Kit *	Quantifiler™ HP DNA Quantification Kit *	Quantifiler™ Trio DNA Quantification Kit *	PowerQuant® System Kit*
Validated real-time PCR instrument(s)	Insta Q96 <sup>®</sup> Insta Q96 <sup>®</sup> Plus Insta Q96 <sup>®</sup> - 6.0	Insta Q96 <sup>®</sup> Insta Q96 <sup>®</sup> Plus Insta Q96 <sup>®</sup> - 6.0	Insta Q96 <sup>®</sup> - 6.0	Insta Q96 <sup>®</sup> - 6.0	Insta Q96 <sup>®</sup> - 6.0	Insta Q96 <sup>®</sup> - 6.0
Channels to be used	FAM – Human VIC – IPC ROX – Passive Reference Dye	FAM – Human VIC – IPC ROX – Passive Reference Dye	FAM – Male VIC – Human NED – IPC ROX – Passive Reference Dye	VIC – Small Autosomal ROX – Large autosomal NED – IPC Cy5 – Passive Reference Dye	FAM – Male VIC – Small Autosomal NED – Large autosomal ROX – IPC Cy5 – Passive Reference Dye	FAM – Human autosomal VIC – Y chromosomal Cy5 – Degradation TAMRA – Internal Positive Control ROX – Passive Reference Dye
Cycling time	~90 min	~90 min	~90 min	~60 min	~60 min	~60 min
Fully integrated system with instrument, data collection, and analysis software	Yes	Yes	Yes	Yes	Yes	Yes
Compatible analysis software		HiGe	enoMB® Forensic-II	DNA Quantification	n Software	
Quality/ degradation index	NA	NA	NA	Yes	Yes	Yes
Human Male: Female Ratio & Sample Inhibitions	NA	NA	Yes	NA	Yes	Yes
Calculation for Dilution and reaction set up in HID kits for downstream application	Yes	Yes	Yes	Yes	Yes	Yes

\* HiGenoMB® Forensic-ID DNA Quantification Software is compatible with Quantifiler™ kits from ABI and PowerQuant® System kits from Promega





## **Products For Forensic Applications At-A-Glance**

Code	Product Name		
Manual Nucleic Acid Purification Kits			
MB524	HiPurA® Forensic Sample Genomic DNA Purification Kit		
MB525	HiPurA® Bone DNA Purification Kit		
MB531	HiPurA® Buccal DNA Purification Kit		
MB504	HiPurA® Blood Genomic DNA Miniprep Purification Kit		
MB554	HiPurA® Multi-Sample DNA Purification Kit		
MB542	HiPurA® Soil DNA Purification Kit		
MB578	HiPurA® Quick Bone DNA Purification Kit		
MB580	HiPurA® Forensic Multi-Sample DNA Purification Kit		
MB522	HiPurA® Sperm Genomic DNA Purification Kit		
MB573	HiPurA® Urine DNA Purification Kit		
MB544	HiPurA® Stool DNA Purification Kit		
Magnetic Bead Bas	sed Extraction Kits		
MB554MPF32200	HiPurA® Multi sample Pre-Filled Plates for Insta NX® Mag32		
MB554MPF96-200	HiPurA® Multi sample Pre-Filled Plates for Insta NX® Mag96		
MB554PC16200	HiPurA® Pre- Filled cartridges for DNA Extraction		
Insta NX® Extraction	on Kits for Forensic Application		
MBIN012	Insta NX® Bone DNA Purification Kit		
MBIN021	Insta NX® Blood RNA Purification Kit		
MBIN001	Insta NX® Blood Genomic DNA Purification Kit		
MBIN003	Insta NX® Tissue Genomic DNA Purification Kit		
MBIN020	Insta NX® Forensic Multi-Sample DNA Purification Kit		

Code	Product Name				
MBIN017	Insta NX® Frozen Blood DNA Purification Kit				
InstaDNA™ Cards/I	InstaDNA™ Cards/Kit				
MBT126	InstaDNA™ card				
MBT137	InstaDNA™ Twin Card				
MBT121	InstaDNA™ Quadricard				
MBT109	InstaDNA™ Kit				
MBT145	InstaDNA™ Twin Card Kit				
MBT158	InstaDNA™ Quadricard Kit				
<b>Identification Kits</b>					
ML189	Blood Identifier Kit				
ML200	Semen Identifier Kit				
ML201	Crime Scene Identification Kit				
ML214	Crime Scene Investigation kit (For sexual assault cases)				
Point Of Care Test	ing (POCT)				
MBLF002	Insta-LF™ Human Blood Point of Care Detection Kit				
Forensic Buffers					
ML127	Stain Extraction Buffer				
ML128	Forensic Buffer				
ML203	1M Sodium Chloride				
ML204	1M Sucrose				
ML205	1M DTT				
PCR Kits					
MBPCR143	Hi-PCR® Horse Probe PCR kit				
MBPCR184	Hi-PCR® Cow Detection Kit (Real-Time Probe Based PCR)				
MBPCR185	Hi-PCR® Buffalo Detection Kit (Real- Time Probe Based PCR)				
MBPCR186	Hi-PCR® Cattle Sex Determination Kit (Real-Time Probe Based PCR)				
MBPCR210	Hi-PCR® Cow-Buffalo Detection Kit (Real-Time Probe Based PCR)				
Software					
MBES01	HiGenoMB® Forensic-ID DNA Quantification Software				
MBES02	HiGenoMB® Primer and Probe Design Software				





# Consumables For Real-Time PCR

PCR Tubes		
CG281	PCR Tubes, Flat lid	
CG282	PCR Tubes, Flat lid	
CG282E	PCR Tube, Flat Lid	
PW1255	PCR Tubes, Thin walled	

PCR Blocks		
PR3	PCR Blocks (Semi-skirt)	
PR5	PCR Blocks (Non-skirt)	
PR19	PCR Blocks (Non-skirt)	

Premium Grade Barrier Tips		
LA749A	Max capacity 10 μL	
LA750A	Max capacity 20 μL	
LA751A	Max capacity 200 μL	
LA859A	Max capacity 1000 μL	

Sealing Films for PCR Blocks		
PR18	Optical Sealing Film 96 well PCR plate	
PR20	Aluminium Sealing Film (for storage)	
PR21	Polypropylene Sealing Film	

PCR S	trips
PR12	8-Strip PCR Tube with dome cap – Clear
PR14	8-Strip PCR Tube without cap – Clear
PR15	8-Strip PCR Tube Flat caps – Clear
PR16	8-Strip optically clear Dome caps for Real-Time PCR
PR17	8-Strip tubes & optically clear flat caps for Real-Time PCR
PR23	8-Strip PCR tubes & optically clear with attached flat caps for Real-Time PCR

Pipettes			
LA611	Varivol II Micropipette-10 (Capacity : 0.5 to 10 μL)		
LA617	Varivol II Micropipette-20 (Capacity : 2 to 20 μL)		
LA618	Varivol II Micropipette-200 (Capacity : 20 to 200 $\mu$ L)		
LA955	$\mu$ Pet Autoclavable Micropipette (Capacity: 0.5 - 10 $\mu$ L)		
LA956	μPet Autoclavable Micropipette (Capacity: 2 - 20 μL)		
LA959	μPet Autoclavable Micropipette (Capacity: 20 - 200 μL)		







## **Technical Parameters of the Product**

Product Name	Insta-Q96® - 6.0		
Product Code	LA1074		
No. of channels	6		
Multiplexing	6 Color		
Sample Capacity	96x0.2 mL Well PCR plate/ tubes, 12x8 (0.2 mL) Strips, optical clear bottom		
Dynamic Range	1~10 <sup>10</sup> Copies		
Excitation Wavelength	300-800nm		
Emission Wavelength	500-800nm		
Detected Flourescence	F1: FAM, SYBR F2: HEX, TET, VIC, JOE, NIC F3: ROX, TEXAS-RED F4: CY5 F5: CY5.5 F6: CY3, NED, TAMRA		
Passive reference dye	ROX or other not required (optional)		
Block Temp. Range	4~105°C (Minimum Increment 0.1°C) Soak Low Temperature, Conservation Function		
Heating / Cooling Rate	4.0°C/s (max)		
Temp. Control Accuracy	≤±0.1°C		
Temp. Fluctuation	≤±0.1°C		
Temp. Uniformity	≤±0.3°C		
Temp. Control Mode	Block / Tube Simulation Mode (Automatic Control Based On Sample Volume)		
Sample Volume Range	5~100 μL		
Gradient Temp. Range	1~36°C		
Hot-Lid Temp. Range	30~110°C (Adjustable Default 105°C), Automatic Hot-Lid		
Flourescence Detection Repeatability	Within 5%		
Scan Mode	Entire Plate or Designated Line		
Run Time	Max 20 Segments for each Program, Max 99 Cycles		
Operation Mode	Continuous		
Scan Period	5.5 seconds		
Feature Function	<ul> <li>Absolute Quantification</li> <li>Automatic Data Analysis</li> <li>Multi-Channel Crosstalk Correction</li> <li>Melt Curve</li> <li>Genotyping</li> <li>Gradient</li> <li>Correction</li> <li>Customized Parameters</li> <li>Compatible with latest forensic human/male DNA testing quantitation kits</li> </ul>		
Operating System	Microsoft: Windows 8/ Windows 10, Software: Excel 2007, 2010, 2013 and Office 365		
PC Configuration	Memory: 4GB RAM, Hard Disk: 500GB, CPU: Intel i3 & latest		
Power Supply	100 - 240V ~ 50/60Hz 600W		
Dimensions (LxWxH)/ Weight	410mm x 386mm x 352mm / 28 kg		
Socket	USB Adapter, Bluetooth Adapter		
Certifications	CE-IVD & ANVISA Approved		

HiMedia Laboratories Pvt. Ltd.

www.himedialabs.com