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1. Technical Specification

The LightCycler® 96 is the latest addition to the LightCycler® Real-Time PCR System family and is the result of more than a decade of Roche experience developing high-performance qPCR systems. The LightCycler® 96 system's ergonomic design features a touch-sensitive screen, intuitive software and flexible connectivity. Together with fast precision thermal cycling and leading-edge optics these features result in an easy-to-use instrument that delivers high-speed and high-quality data.

• Instrument hardware:

1. Block Configuration

o The LightCycler® 96 real-time PCR instrument is a <u>96-well format</u> instrument, compatible with 96-well plates and 8-tube strips and supports the use of reaction volumes in the range of 10-50 μl per well.

O Thermal cycling is peltier-based with fast ramp rates (4.4°C for heating and 2.2°C for cooling) and accurate and homogenous temperature control (±0.2°C of programmed target temperature; Tm uniformity range across the block of 0.4°C with standard deviation < 0.1°C). The instrument permits the use of temperature gradients during a run with a maximum span of 20°C across the block. Collectively these features contribute to the LightCycler® 96 being a fast speed and accurate instrument, with flexibility for assay optimisation afforded by the temperature gradient.

2. Optics

- o The LightCycler® 96 has a high-power broad spectra white LED light source and fibre-optic acquisition system with 4 detection channels (paired excitation and emission filters). The LED light source has a working life in the region of 10,000 working hours meaning that downtime to permit bulb replacement is diminished.
- o The excitation/emission channels available are (in nm) 470/514 (SYBR, FAM, ResoLight), 533/572 (VIC, Hex, Yellow555), 577/620 (Red610, Texas Red) and 645/697 (Cy5). These are compatible with all mainstream PCR dyes including intercalating dyes (e.g. SYBR Green I, ResoLight) and TaqMan® probes (e.g., UPL). The LightCycler® 96 does not require passive reference dyes such as ROX to be included in the wells so up to 4-plex assays can be conducted on the system. Furthermore the channels are pre-calibrated so users are not required to spend time optimising for colour compensation.
- The fibre-optic head permits simultaneous data acquisition from every well at the exact same time therefore greatly reducing assay run times,

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particularly those assays employing higher resolution programmes (e.g. melt curve analysis and high-resolution melting) or multiple fluorescence channels. This optics system is designed with no moving parts such as a scanning head, resulting in a system that is highly accurate and extremely reliable, minimising any potential system downtime.

- Instrument Dimensions and Laboratory requirements for the system: The LightCycler® 96 is a compact instrument with a footprint of 40cm wide by 40cm deep and stands 53cm high so does not require a large area of bench space. The instrument weighs approximately 25kg and the bench requirements are access to a single 3-pin UK plug socket for the power cable and access to a network port (optional: only required if the instrument is to be networked). It is therefore anticipated that positioning the system within the laboratory will not require a great deal of time or bench re-organisation.
- Instrument Software, Interface and Connectivity:
 - 1. LightCycler® 96 on-board application software
 - The LightCycler® 96 has a large 10.4" touch-sensitive screen (Resolution 800 x 600). The on-board application software is intuitive to use and permits the user to access and change the instruments configuration settings, program experiments from scratch/templates or amend pre-existing programs prior to executing the experiments.
 - No well-specific information is required to get an experiment running, thus enabling even a novice user to program an experiment from scratch and start it within a few minutes.
 - Experiment programs can also be imported to the instrument by USB2.0, local network (LAN) and/or 1:1 connection between the instrument and a computer. Additionally up to 50 experimental files can be stored on the instruments built in hard-drive.
 - o By networking the instrument it is possible to set-up email notifications, whereby on completion of the experiment the end user will receive a notification message with the option of having the experimental file attached. Collectively these features provide flexibility to suit the preferences of different users.
 - 2. LightCycler® 96 analysis software features
 - o The LightCycler® 96 system and software together can run a broad range of PCR-based applications including gene-detection, gene expression and SNP genotyping and the simple and intuitive analysis software provides the capacity to analyse data using Absolute Quantification, Relative Quantification, Tm Calling (melt curve analysis), Endpoint Genotyping, High Resolution Melting and Qualitative detection.
 - o Analysis software is supplied to permit the analysis of LightCycler® 96 experimental files and to design experiments prior to transferring to the instrument for execution. The LightCycler® 96 analysis software is licence free, meaning that instrument users can install and run it on as many computers as they require.



- o The LightCycler® 96 analysis software allows run reports to be generated in html format, providing separate windows for run information, run profile, sample information, raw data and a window for to each separate analysis.
- When an instrument software update becomes available, the instruments owners will be notified and the updated software version made available at no extra charge.
- 3. Computer requirements for running LightCycler® 96 analysis software To install and run the LightCycler® 96 Application Software, the computer must satisfy the following minimum requirements:

o Processor: Intel Core 2 duo 2.4 GHz

o Memory: 2 GB o Hard disk: 250 GB

o LAN: RJ45 Ethernet (100 MBit)

o USB: USB 2.0

o Display resolution: 1280 * 1024

- Operating system: Microsoft Windows XP or Microsoft Windows 7, 32bit configuration. (N.B. detailed in product bulletin 036/2013; 'In addition the software was also tested for Microsoft Windows 8 without any noticeable limitations').
- o Microsoft .NET Framework 4.0 installed
- o Setting for regional and language options: English (USA)

4. Connectivity

o The LightCycler® 96 is fully networkable (LAN) and either networking or connecting the instrument directly to a computer permits the live monitoring of assays and the transfer of experimental data files. The LightCycler® 96 can also be run as a stand-alone system whereby file transfer using a USB2.0 compatible flash-drive is possible. As such, connectivity is flexible and will allow individual users to choose what methods best suits their preference.

2. Installation, commissioning and training on the system

Installation of the LightCycler® 96 is a quick process, taking approximately 30 minutes. Waste packaging materials will be removed from site following the installation. Installation and training will be organised to fit around the employees work schedules and is included in the purchase price. Should training sessions for new starters or refresher courses be required further down the line these can be organised on an as and when basis, at no extra cost and to a maximum of 2 sessions per year. The installation and commissioning of the LightCycler® 96 and the training on its use will be conducted by the Sales Specialist for Scotland, Peter Vesey. Peter joined Roche Diagnostics in October 2012 having previously worked as a Post-doctoral researcher at the University of Buffalo. Peter draws on many years of experience of using and developing qPCR assays, having employed qPCR during his own research and building on his knowledge and training expertise both in the field and through product training with Roche over the last 2.5 years.