Real-time PCR Detection system

Equipment /Instrument Details

Make : BIO RAD Labs

Model : CFX96 (C1000 Thermal cycler)

Important features

Precise selection of wells, dyes and volume of reaction mixture. Selection of analysis mode in a more efficient mode. Edit plate set up and viewing the generated results. Option for exporting the data into publishable formats like MS word and image.

Specifications

Thermal Cycler:	
Chassis	C1000 Touch
Maximum ramp rate	5ºC/sec
Average ramp rate	3.3ºC/sec
Heating and cooling method	Peltier
Lid	Heats up to 105 ºC
Temperature: **Range** **Accuracy** **Uniformity**	 0 to 100 ºC ±0.2 ºC of programmed target at 90 ºC ±0.4 ºC well-to-well within 10 sec of arrival at 90 ºC
Gradient **Operational range** **Programmable Span**	 30 - 100 ºC 1 - 24 ºC
Optical Detection: **Excitation** **Detection** **Range of excitation/Emission**	 6 filtered LEDs 6 filtered Photodiodes 450-730 nm
Wavelengths: **Sensitivity** **Dynamic Range**	 Detects one copy of target sequence in human genomic DNA 10 orders of magnitude
Scan time: **All channels** **Single channel fast scan**	 12 sec 3 sec

Usages

Quantitative Reverse Transcriptase Real-time PCR is a laboratory technique in molecular biology for the detection and quantitation of an amplified PCR product based on incorporation of a fluorescent reporter dye; to calculate the absolute and relative gene expression in a given sample. Can also be used as gradient and conventional PCR purposes.

Working Principles

The principle of real-time detection system is based on detection of PCR products which is enabled by the inclusion of a fluorescent reporter molecule in each reaction well that yields increased fluorescence with an increasing amount of product DNA. The principle is based on fluorescence chemistries employed for this purpose include DNA-binding dyes and fluorescently labeled sequence-specific primers or probes.

The fluorescence detection modules are used to monitor the fluorescence signal as amplification occurs. The measured fluorescence is proportional to the total amount of amplicon; the change in fluorescence over time is used to calculate the amount of amplicon produced in each cycle.

Application

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User instructions (including sampling instructions)

Setting up of qPCR reaction mixture:

- All the qPCR reactions must be performed as per MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines.
- Always prepare PCR reaction mixture under aseptic environment using nuclease free tubes and barrier tips avoiding DNA contamination. Never prepare mixtures involving NTC and DNA of interest at the same facility.
- Briefly vortex and spin all the contents of qPCR master mix to avoid air bubbles.
- Every time you run the qPCR, include positive DNA control, negative DNA control and No template control.
- Always standardize the primer annealing temperatures in conventional PCR before proceeding for real-time thermal cycler.

Calibration

Calibration analysis has to be performed every six months using BIO-RAD calibration kit. Depending upon the reaction vessel to be used, calibration has to be performed accordingly for plates or strips with domed and flat caps.

Suggested charges including GST

S.No.	Type of Analysis	Industry	University	ICAR Institutes	Other	Unit
1	Absolute Gene expression	1000	700	500	1000	*Rates are per hour
2	Relative Gene Expression	1000	700	500	1000	*Rates are per hour
3	Primer amplification efficiency	1000	700	500	1000	*Rates are per hour

The rates are tentative and exclusive of the consumables

Contact Person
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The Demand Draft should be in favour of "The Director, CARI, Bareilly (UP)"
Letter, DD & Samples may be sent to "The Director, CARI, Bareilly (UP)-243122"

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