RE-IMAGINE
Real-Time PCR

Azure Cielo™
Real-Time PCR

Innovation Driving Performance

azure biosystems
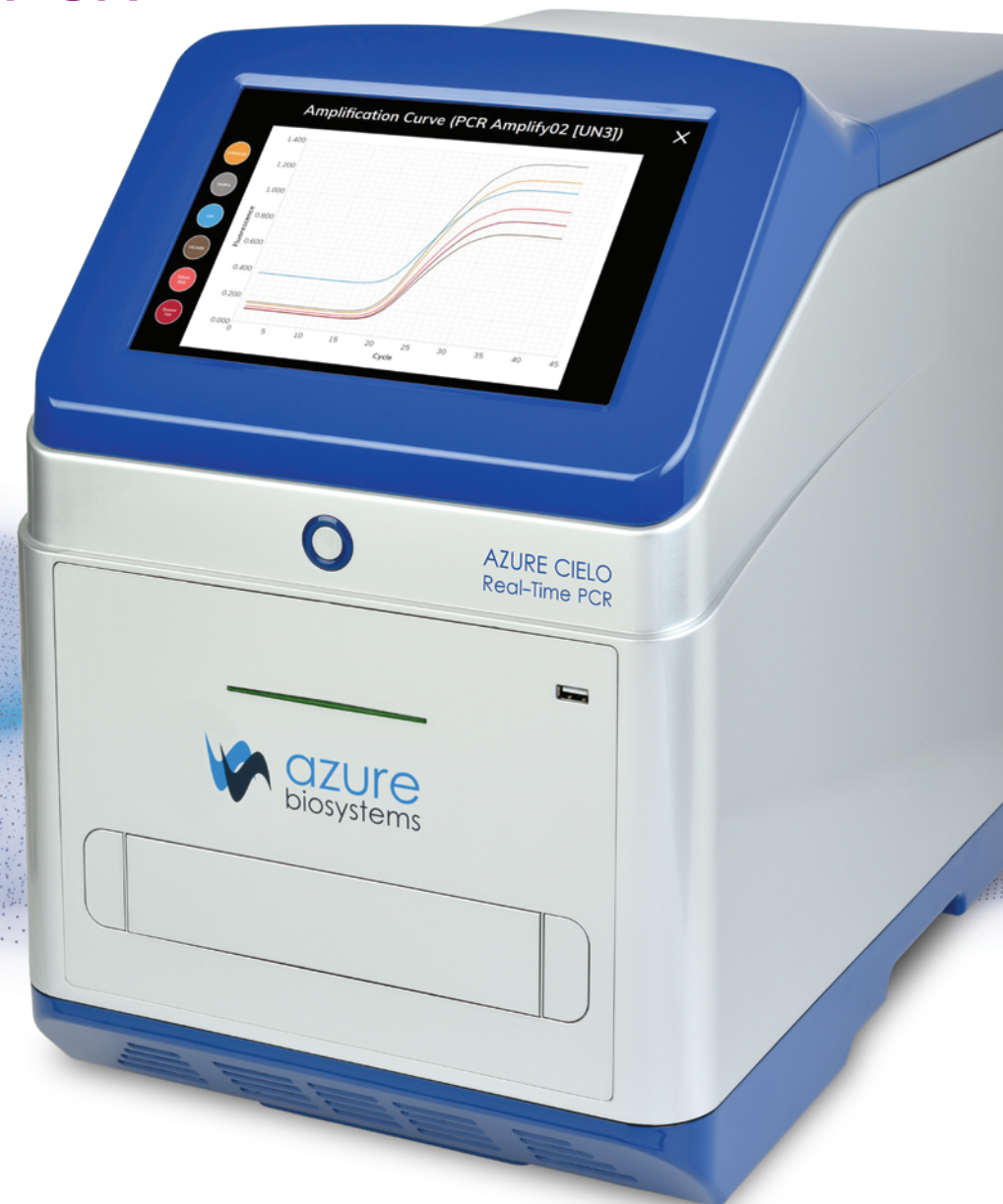
Designed to deliver the highest level of performance, Azure Cielo Real–Time PCR brings out the best in your data

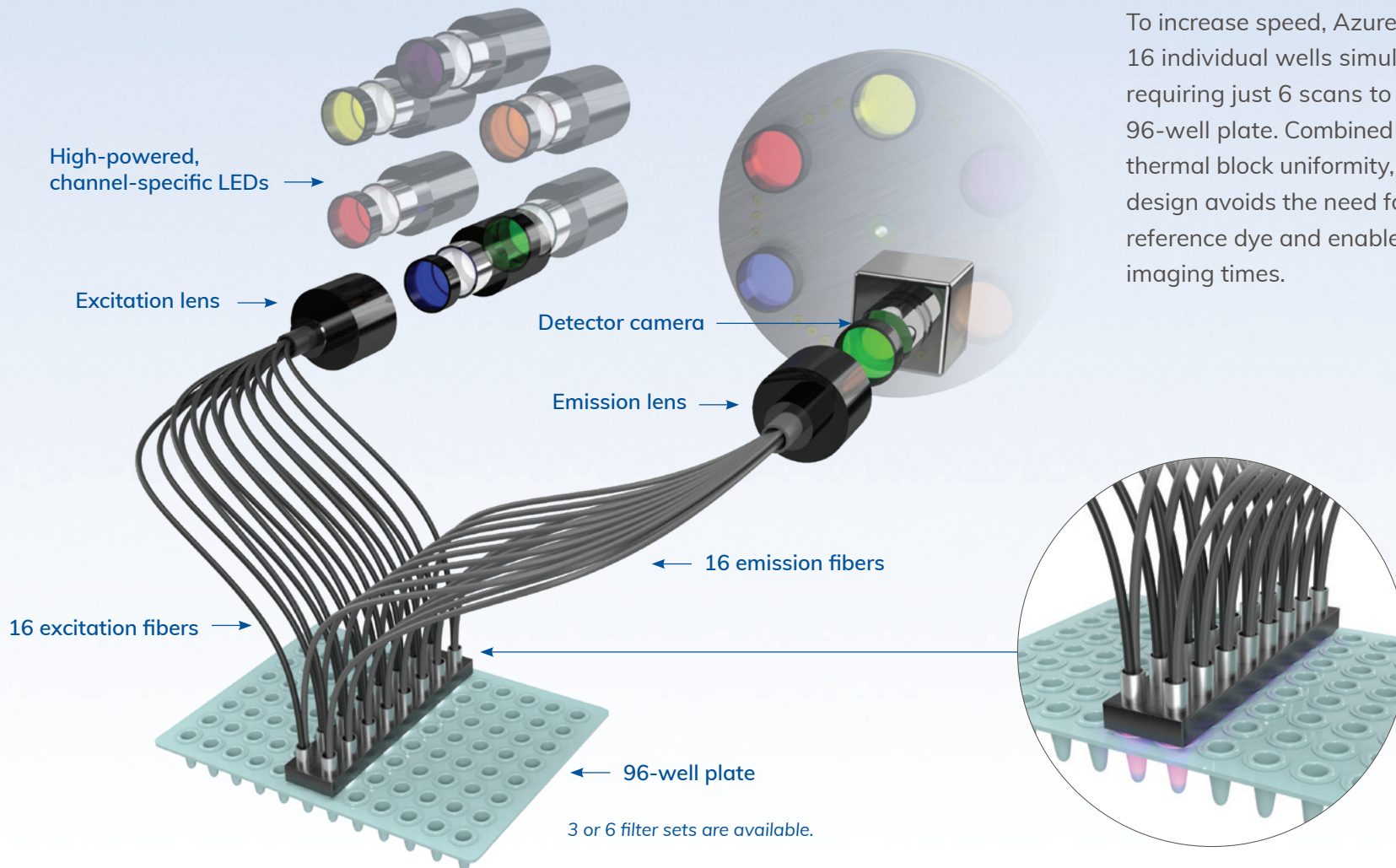Offering innovative optics and technology for high-performance real-time PCR:

▸ Flexibility through innovation

▸ Sensitivity by design

▸ Engineered for faster run times

▸ Built for reproducibility

▸ Simplicity through software

Reliable system packaged with innovative and high-performance optical technology

Azure Cielo was designed around individual well scanning, comprising of two sets 16 fiber optics. Channel-specific excitation and emission light emitting diode (LED) light sources ensure that specific wells on the qPCR plate are illuminated, increasing sensitivity and reducing background noise.

To increase speed, Azure Cielo images 16 individual wells simultaneously, requiring just 6 scans to scan an entire 96-well plate. Combined with high thermal block uniformity, Azure Cielo's design avoids the need for a passive reference dye and enables faster imaging times.

High-powered, channel-specific LEDs →

Excitation lens →

Detector camera

Emission lens →

16 emission fibers

16 excitation fibers

96-well plate

3 or 6 filter sets are available.

Flexibility through innovation

Up to 6 fluorescent channels allow for flexible dye usage and broad application. Additionally, Rox isn't required, so you can use it as a probe.

SIX TARGET MULTIPLEX

Standard Curves
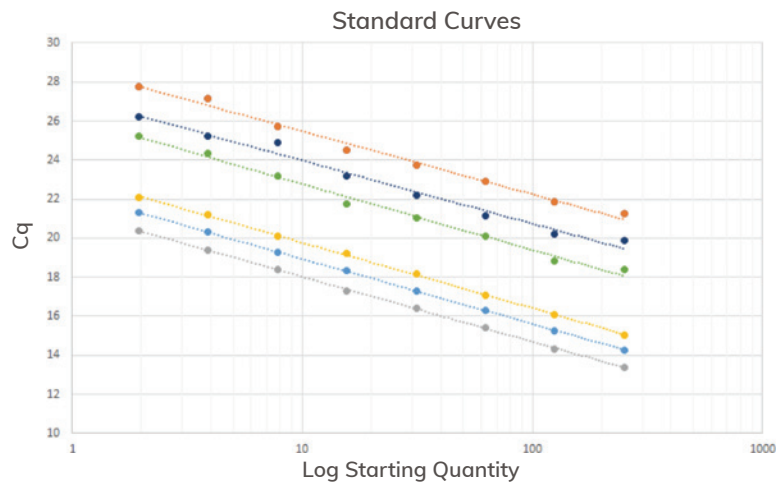
Report Dye	Gene name	Efficiency	R^2	Slope	Y-intercept
FAM	RRP36	103.582	0.959	-3.239	28.684
HEX	ACTB	100.456	0.995	-3.311	21.281
TAMRA	RNASE-P	97.016	0.995	-3.396	22.157
Texas Red	GAPDH	100.219	1.000	-3.317	22.307
Cy5	TBP	96.811	0.993	-3.401	26.023
Cy5.5	EF1a	97.224	0.993	-3.390	23.173

Linear dynamic range in 6-plex one-step reverse transcription qPCR reactions. Six targets across eight orders of magnitude (250ng to 1.95ng** total input RNA) were amplified using BioRad Multiplex Supermix on an Azure Cielo 6 Real-Time PCR system. The results showed exceptional performance, with high efficiencies and linearity over a wide dynamic range.

SINGLEPLEX QPCR REACTIONS WITHOUT ANY CROSSTALK
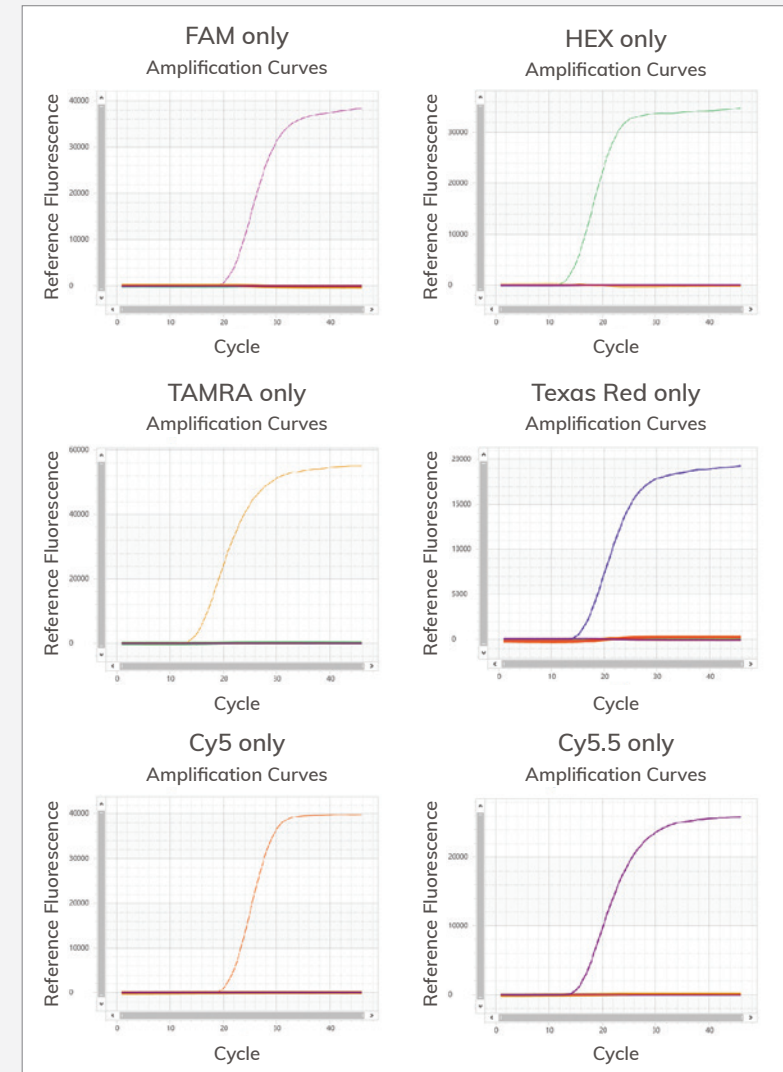
Multiplex qPCR reaction were scanned for all 6 channels in an Azure Cielo Real-Time PCR system. Single channel amplification curves were as expected, with no signal leakage or crosstalk.

Broad application compatibility

The Azure Cielo Real-Time PCR system is available in either a 3-channel or 6-channel configuration and is compatible with a wide spectrum of qPCR dye-based and probe-based chemistries.

Dyes/Probes	Azure Cielo 3 Real Time PCR system	Azure Cielo 6 Real Time PCR system	Filter wavelength (nm)	
			Excitation	Emission
• SYBR® Green • EvaGreen™ • FAM™	✔	✔	475 ± 14	524 ± 12
• VIC® • HEX™ • JOE™ • CAL Fluor® 540 • CAL Fluor® Orange 560	✔	✔	527 ± 10	565 ± 12
• TAMRA™ • Cy3		✔	537 ± 13	583 ± 11
• ROX™ • TEX®615, • CAL Fluor® Red 610		✔	572 ± 7.5	623 ± 12
• Cy®5 • Quasar®670, • Liz® • Mustang Purple®	✔	✔	623 ± 12	676 ± 18.5
• Cy®5.5 • Quasar 705		✔	655 ± 7.5	711 ± 12.5

Custom dyes and probes are compatible based on system configuration and are easily calibrated using an intuitive one-step procedure.

Sensitivity by design

Efficient illumination and detection of each well provides exceptional specificity and precision.

Unlike systems that use whole-plate imaging, no excitation light falls outside of the well when using the Azure Cielo Real-Time PCR system. This reduces potential background that could mask fluorescent signals from qPCR samples. Additionally, in contrast to whole-plate detection, the Azure Cielo Real-Time PCR system provides uniform illumination within each well, and from well to well, increasing the excitation efficiency of the qPCR reaction.
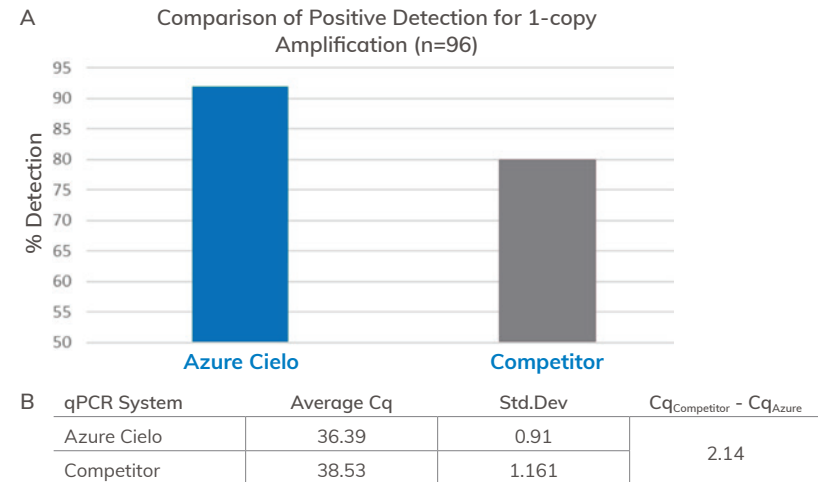
Azure Cielo   **Competitor**

Single illumination light sources can deliver light both in and out of the wells, resulting in higher background, and less light hitting the sample. Azure Cielo uses fiber optics to directly deliver light to each indivdual well.

RELIABLY DETECT 1.5-FOLD DIFFERENCES IN TARGET AMOUNT
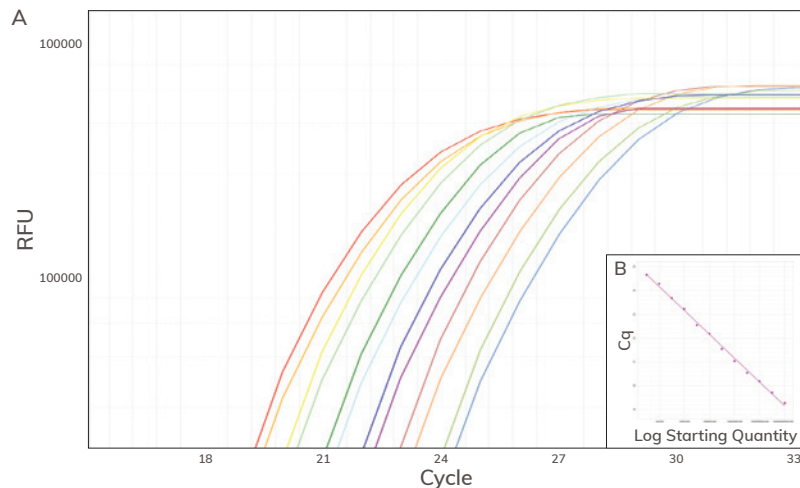
Reliable detection of 1.5-fold differences. Figure A. Amplification curves for assays of GAPDH in a dilution series of human reference cDNA (n=3). Assays were conducted using BioRad™ Sso Advanced SYBR Green Mix®. Figure B shows the standard curve obtained by plotting Cq values vs the amount of template DNA (copies/μl). R^2=0.998, efficiency = 99.89%.

## DETECT CQS EARLIER, EVEN FOR SINGLE COPIES OF YOUR TARGET GENE

qPCR System	Average Cq	Std.Dev	$Cq_{Competitor}$ - Cq_{Azure}
Azure Cielo	36.39	0.91	2.14
Competitor	38.53	1.161	

Superior single copy detection. Real-Time PCR was carried out using BioRad Prime PCR Probe Assay for GAPDH, 1 copy of Human Reference GAPDH DNA Template and Biorad Multiplex Supermix. A) Comparison of amplification detection in Azure Cielo Real-Time PCR system and Competitor shows higher rate of 1-copy detections. B) Average Cq obtained from Azure Cielo System is 2 cycles earlier than Competitor. Cqs from the above experiment were averaged.

Engineered for faster run times

Simultaneous scanning of 16 wells provides faster scanning speed compared to individual well scanning technology.

FAST RUN TIMES FOR FULL PLATE PROTOCOLS
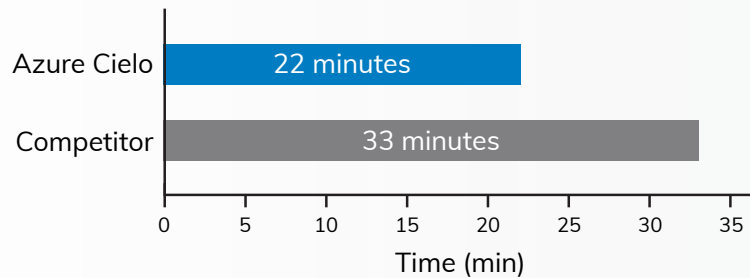
A commercially available fast qPCR kit was run, using SYBR Green. Protocol as follows: 95°C, 2 minutes, 40 X (95°C 1 second, 60°C 1 second).

## SIMULTANEOUS SCANNING FOR QUICK IMAGING

Single well imaging has many advantages but scanning each well individually can reduce the overall cycle speed. By scanning 16 wells in parallel, the Azure Cielo Real-Time PCR system requires just 6 scans for an entire 96 well plate, with 6-channel entire plate scan taking only 9 seconds.
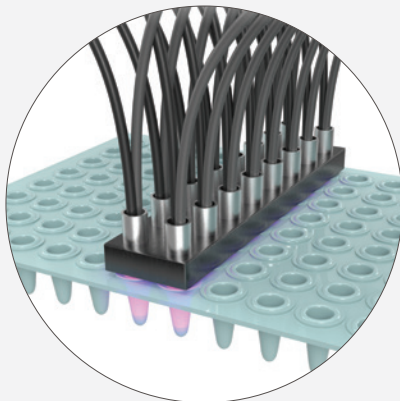
The Azure Cielo 6 Real-Time PCR system moves 6 times in 9 seconds to scan an entire 96-well plate.
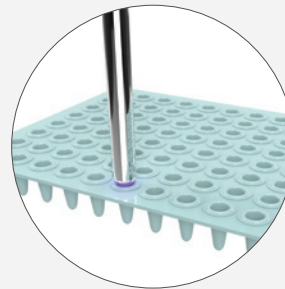
Single well detector-based (non-fiber optic) systems must move 96 times to read each channel.
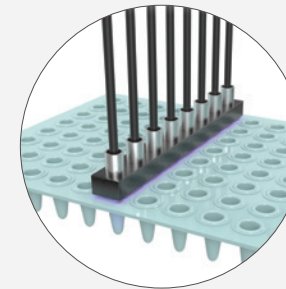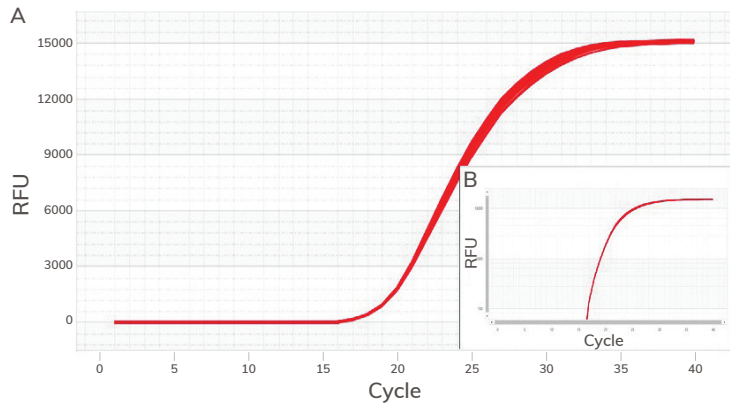
8 well channel systems have to move the scan head 12 times to read each channel.

Built for reproducibility

High-quality engineering includes Peltier-based thermal block system designed for superior thermal cycling and unmatched optical design to ensure every run is accurate and reproducible.

TIGHT REPLICATES THROUGH SUPERIOR INTER-WELL UNIFORMITY

Unparalleled uniformity. Amplification curves for 96 replicates shown on a linear plot (A) and a logarithmic plot (Figure B). In each well, 10^5 copies of GAPDH template were amplified in the presence of GAPDH primers and BioRad™ Sso Advanced SYBR Green Mix®. Average Cq = 19.1, Coefficient of variance (Cv)= 0.002.
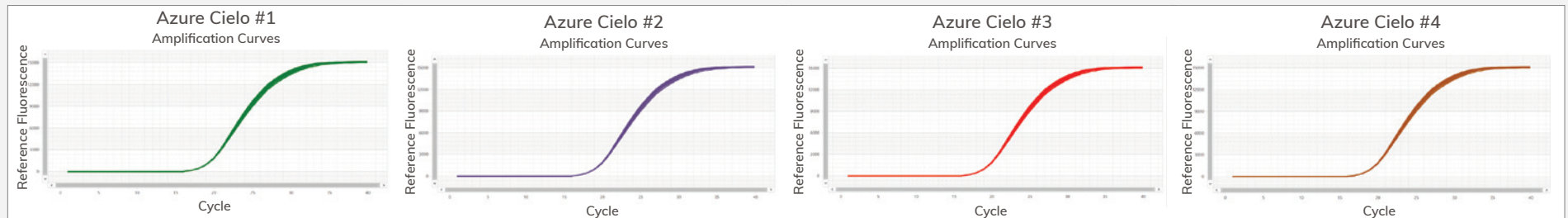
ROBUST DESIGN ENSURES REPRODUCIBILITY FROM RUN TO RUN

Cielo's optics and thermal block are designed to deliver the same uniformity and reproducibility over at least 1000 qPCR experiments, giving you confidence in the reliability of the instrument.

Cq vs Number of qPCR experiments
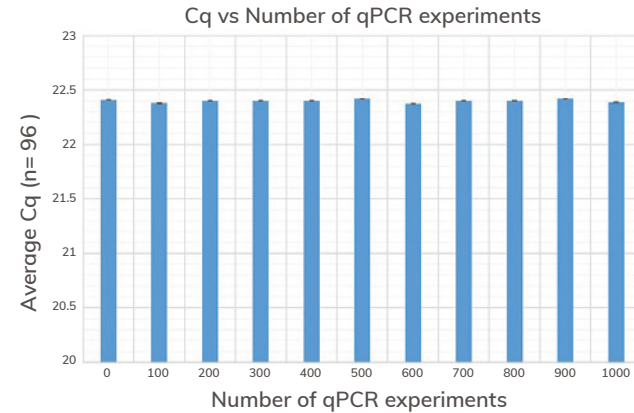
Robust design. After sequential sets of 1000 continuous qPCR experiments, a GAPDH qPCR assay was performed and Cq values were recorded at every 100 experiments points. Average Cq in each assay = 22.4, ±0.01.

INSTRUMENT TO INSTRUMENT UNIFORMITY

Azure Cielo #1 Amplification Curves

Azure Cielo #2 Amplification Curves

Azure Cielo #3 Amplification Curves

Azure Cielo #4 Amplification Curves

Reproducible data on four separate Azure Cielo instruments using Human GAPDH assay and SYBR Green Master Mix. Average Cq = 20.11, SD= 0.002

OPTICAL DESIGN ELIMINATES NEED FOR ROX

Azure Cielo Real-Time PCR system's efficient well-based scanning technology scans multiple data points within each well eliminating the need for normalization dyes such as ROX.

Amplification Curves
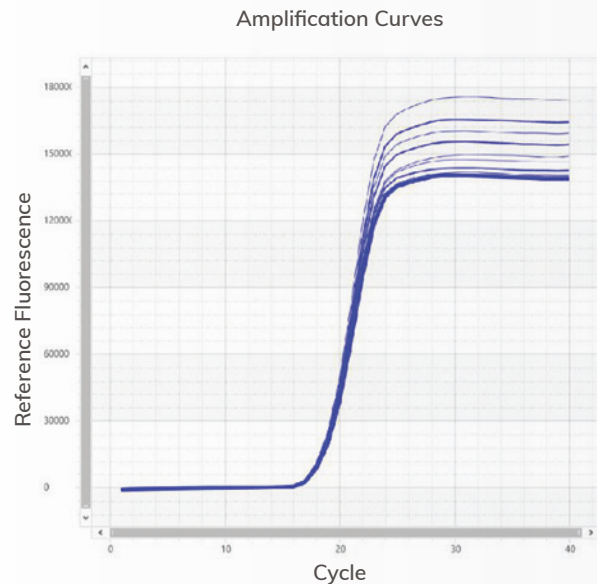
Optical design eliminates need for ROX. Amplification curves generated using human genomic DNA template along with human GAPDH primers and a no ROX SYBR Green qPCR mix.

Replicate	Fluorescence	Cq	Cq Mean	Cq Standard Deviation	Cq CV
1		17.54			
2		17.57			
3		17.55			
4		17.57			
5	SYBR Green (NO ROX)	17.56	17.54	0.02	0.13%
6		17.54			
7		17.52			
8		17.52			
9		17.51			
10		17.51			

Reactions with no passive reference dye added. Cq values and respective statistical data obtained from ten replicate wells using the Azure Cielo Real-Time PCR system. The Cq values obtained from ten replicate wells and the respective statistical data.

Azure Cielo acquires approximately 100,000 data points/well using total well detection technology.

~100,000 pixels/well   1 pixel/well

Azure Cielo   **Competitor**
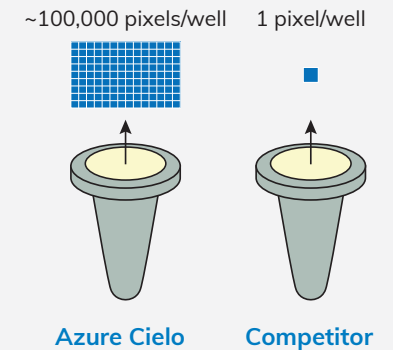
Competitive systems can acquire only 1 pixel/well compared to Azure Cielo Real-Time PCR systems that can capture approximately 100,000 pixels/well enabling accurate, reproducible and sensitive representation of fluorescent intensities from each qPCR well.

Simplicity through software

A compact system with integrated touch screen and intuitive, protocol-based software.
Built-in Wi-Fi, ethernet and USB allow for remote control and make transferring data easy.

AZURE CIELO MANAGER

Azure Cielo Manager is PC-compatible software designed to let you set up,
run and analyze your real-time PCR data from your personal computer.

Key features include:

- Data analysis modes for qPCR with dye/SYBR,
  quantitative PCR with probe, allele discrimination with
  probe, comparative quantitation

- Intuitive plate map design allows users to assign well ID as
  standards, unknowns, calibrators, negative controls, NTC, etc.

- Easily set up and monitor thermal profiles including
  temperature, time and plate scan for each step

- View real-time qPCR graphs during an active
  experiment to monitor real-time reactions

- Post-run summary and analysis with ability to view
  statistical data in the form of charts, curves and graphs

- View a complete and easily customizable report of
  the qPCR experiment that can be readily exported

- Easily copy/paste data or graphs into Paint, Microsoft
  Office or other supported applications as needed

- Export data to MS Office, PDF or in MIQE
  preferred RDML (1.0, 1.1, 1.2) formats

Designed by scientists, for scientists
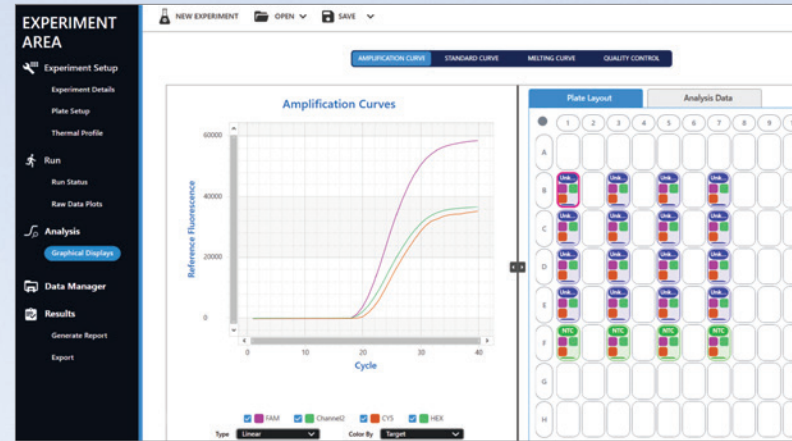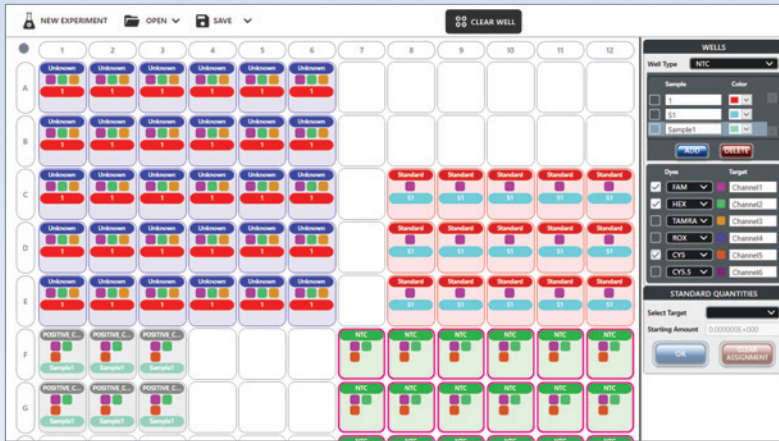
Quickly set up and confirm plate layout. Each well is color coded by sample type, biological replicate, and technical replicate. Well type and standard curve values are also easily visible.

View each individual amplification trace by target or sample. Easily adjust threshold values by dragging the threshold line, or typing in the threshold value.

View 2D "heat map" of samples to quickly understand your data.

Receive end of experiment notification and data file via email.

Specifications	Azure Cielo 3 Real-Time PCR system	Azure Cielo 6 Real-Time PCR system
Part number	AIQ030	AIQ060
Product description	96-well Real-Time PCR instrument with 10.3" touchscreen interface, 3 dye channel filters	96-well Real-Time PCR instrument with 10.3" touchscreen interface, 6 dye channel filters
Sample capacity (wells)	96	
Compatible plates/tubes	96-well plates & tubes and 8 or 12-well strips & single tubes	
Reaction volume	5–150μL	
Excitation source	LED, Excitation range: 450–580nm and 610–700nm	LED, Excitation range: 450–730nm
Detection channels	3	6
Multiplexing	Up to 3 targets	Up to 6 targets
Thermal element	Peltier	
Gradient	12 Independent temperature zones with a maximum range of 40°C	
Lid temperature	Heats up to 105°C	
Reaction temperature	4°C to 99.9°C	
Max. block ramp rate	6°C/sec	
Avg. sample ramp rate	4°C/sec	
Temperature uniformity	±0.2°C	
Temperature accuracy	±0.1°C	
Custom dye/chemistry	✔	
Chemistry capability	Fast/Standard	
Detection sensitivity	1 copy	
Sensitivity	Detect differences as small as 1.5-fold in target quantities in singleplex reactions	
Onboard memory	32GB equivalent to 20,000 experiments	
Connectivity	USB, Wi-Fi, Ethernet	
Electrical approvals	CE Certified	
Footprint (WxDxH)	12" x 20" x 17" (30.5 x 50.8 x 43.2 cm)	

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