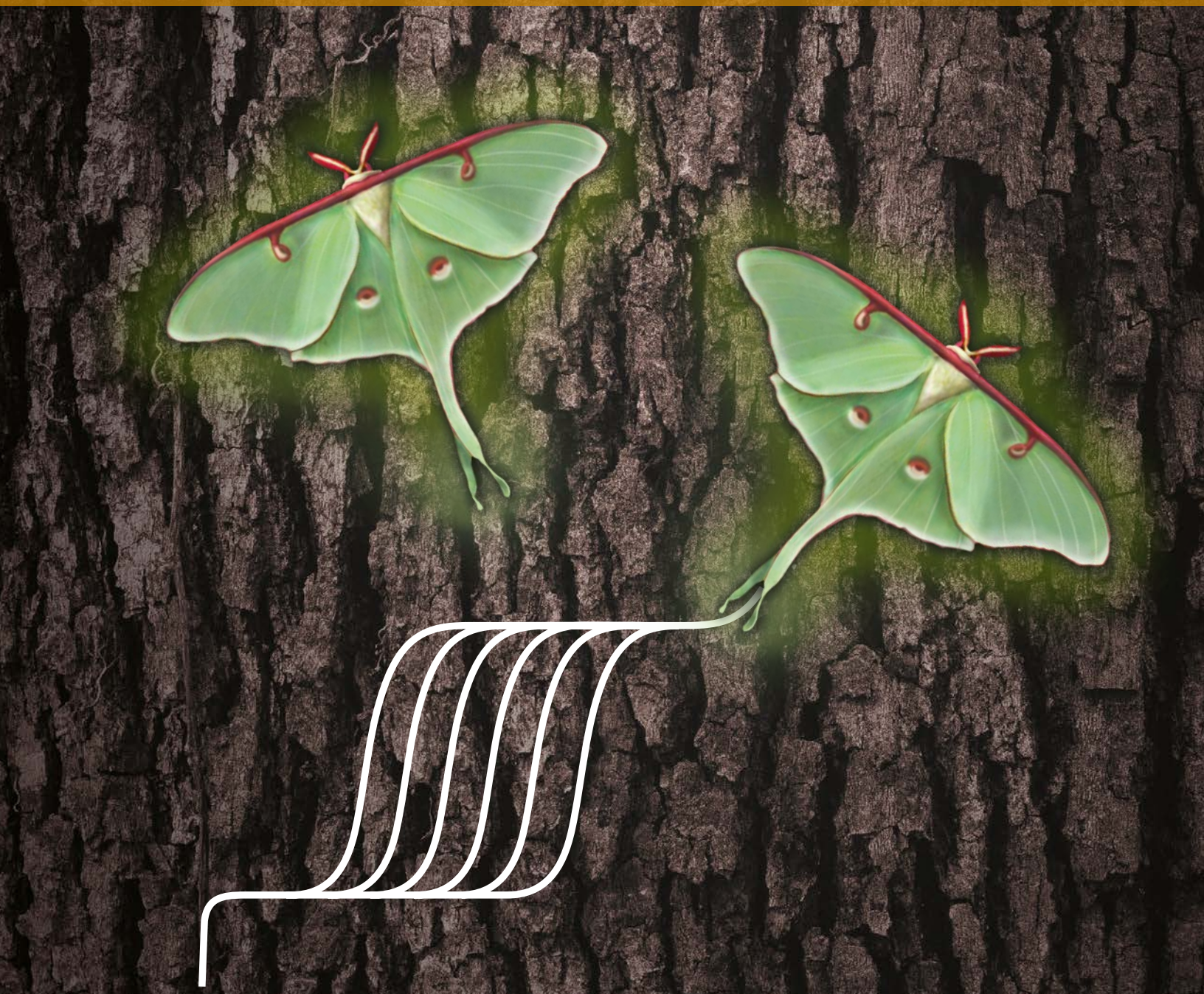
Now includes
LunaScript® Multiplex One-Step RT-PCR Kit

Luna® Universal qPCR & RT-qPCR

LIGHTING THE WAY™



NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

Lighting the Way

Fluorescence-based quantitative polymerase chain reaction (qPCR) is the gold standard for the detection and quantification of nucleic acids due to its sensitivity and specificity. Luna products are optimized for qPCR or RT-qPCR, and are available for either intercalating dye or probe-based detection methods.

Each Hot Start *Taq*-based Luna qPCR master mix has been formulated with a unique passive reference dye that is compatible across a wide variety of instrument platforms, including those that require a ROX reference signal. This means that no additional components are required to ensure machine compatibility. The mixes also contain dUTP, enabling carryover prevention when reactions are treated with NEB's Antarctic Thermolabile UDG (NEB #M0372). The Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) includes both components in the master mix.

The Luna Cell Ready Lysis Module and kits are designed for direct RNA quantitation from cell lysate, bypassing traditional RNA extraction and purification steps. Coordinated cell lysis, RNA release, and genomic DNA removal is achieved in a 15 min protocol.

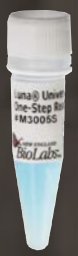
For two-step RT-qPCR, the LunaScript RT SuperMix Kit offers a fast (13 min), robust, and sensitive option for cDNA synthesis upstream of your Luna qPCR experiment. The supermix contains a blue tracking dye, allowing you to easily track your samples throughout the RT-qPCR workflow.

Find the right Luna product for your application

1 Select your target

2 Select your detection method

	Dye-based	Probe-based
Genomic DNA or cDNA	Luna® Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)
Purified RNA **One-Step RT-qPCR**	Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	Luna Universal Probe One-Step RT-qPCR: • Kit (NEB #E3006) • Kit (no ROX) (NEB #E3007) • 4X Mix with UDG (NEB #M3019)
Two-Step RT-qPCR	LunaScript® RT SuperMix Kit (NEB #E3010) + Luna Universal qPCR Master Mix (NEB #M3003)	LunaScript RT SuperMix Kit (NEB #E3010) + Luna Universal Probe qPCR Master Mix (NEB #M3004)
RNA from cell lysate	Luna Cell Ready One-Step RT-qPCR Kit (NEB #E3030)	Luna Cell Ready Probe One-Step RT-qPCR Kit (NEB #E3031)



Make a simpler choice

- Non-interfering, visible tracking dye eliminates pipetting errors
- LunaScript Multiplex One-Step RT-PCR Kit (NEB #E1555) combines Luna WarmStart® Reverse Transcriptase (RT) and Q5® Hot Start High-Fidelity DNA Polymerase for superior multiplexing and sensitive detection

Optimize your One-Step RT-qPCR

- Luna WarmStart RT is a novel, thermostable RT with improved performance
- WarmStart RT paired with Hot Start *Taq* enables room temperature setup and stability
- 4X concentration option (NEB #M3019) allows for more sample input, increased sensitiivity

Speed up your Two-Step RT-PCR

- LunaScript RT SuperMix Kit is optimized for first strand cDNA synthesis with a fast 13-minute protocol
- Primer-free option (NEB #E3025) enables flexibility of primers used for optimal cDNA synthesis

Learn more at LUNAqPCR.com

DOWNLOAD THE NEB AR APP*

Find an overview of qPCR.

*see back cover for details

We tested plates and plates of reactions so you don't have to.

Evaluating qPCR results: capturing performance as "dots in boxes"

NEB has developed a method to better evaluate the large amount of qPCR data generated in an experiment. The output of this analysis is known as a dot plot, and captures the key features of a successful, high-quality qPCR experiment as a single point. This method of analysis allows many targets and conditions to be compared in a single graph.

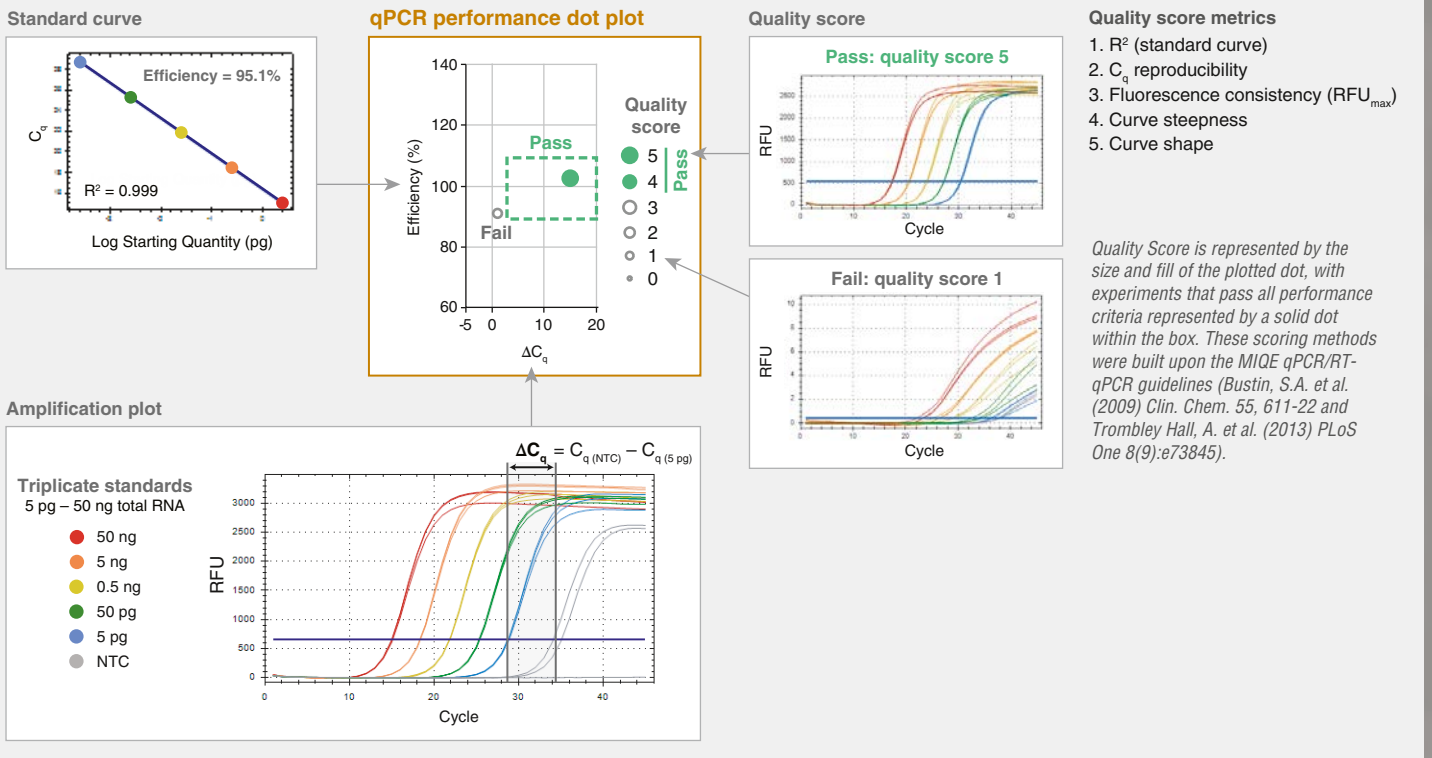
For each experiment, triplicate reactions are set up across a five-log range of input template concentrations (Amplification plot, bottom-left). Three non-template control (NTC) reactions are also included, for a total of 18 reactions per condition/target. Efficiency (%) is calculated (Standard plot, top-left) and is plotted against ΔC_q (dot plot, top-center), which is the difference between the average C_q of the NTC and the lowest input. This parameter captures both detection of the lowest input and non-template amplification.

Acceptable performance criteria are defined as an Efficiency of 90–110% and a ΔC_q of ≥ 3 (green box – pass).

Other performance criteria are captured using a 5-point quality score (Quality score metrics, top-right). Included are:

1. Linearity of amplification, as indicated by the R^2 standard curve
2. Reproducibility, as indicated by the consistency of triplicate C_q values for each input concentration
3. Fluorescence consistency, as indicated by similar endpoint fluorescence (RFU_{max})
4. Curve steepness
5. Sigmoid curve shape

Breaking it down: how we translate qPCR data into "dots in boxes"



Quality score metrics

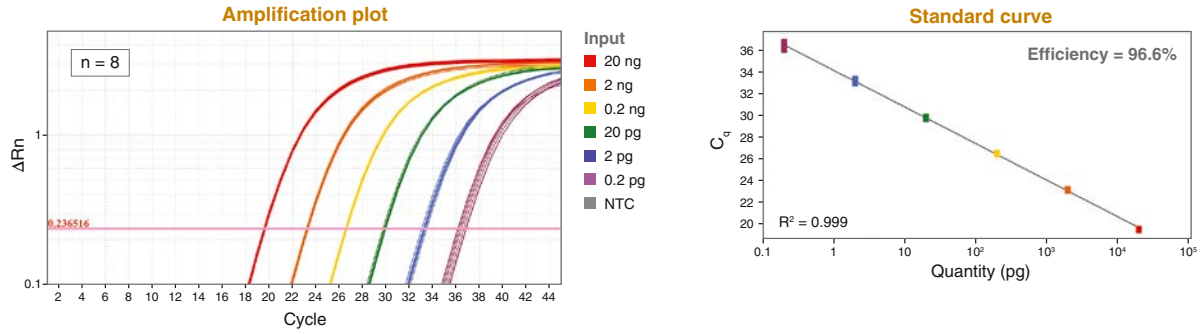
1. R^2 (standard curve)
2. C_q reproducibility
3. Fluorescence consistency (RFU_{max})
4. Curve steepness
5. Curve shape

Quality Score is represented by the size and fill of the plotted dot, with experiments that pass all performance criteria represented by a solid dot within the box. These scoring methods were built upon the MIQE qPCR/RT-qPCR guidelines (Bustin, S.A. et al. (2009) Clin. Chem. 55, 611-22 and Trombley Hall, A. et al. (2013) PLoS One 8(9):e73845).

Experience Best-in-class Performance for

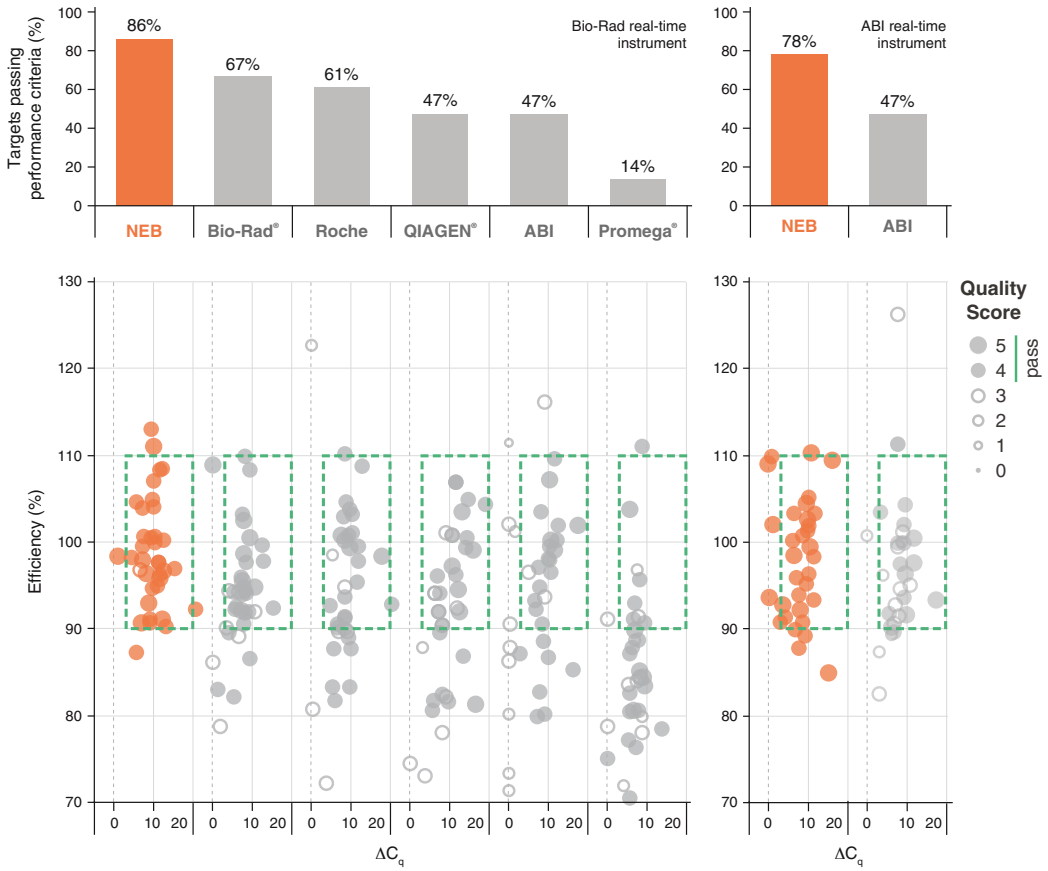
All NEB products undergo rigorous testing to ensure optimal performance, and Luna is no exception. We took into consideration numerous important traits when evaluating qPCR, including specificity, sensitivity, accuracy and reproducibility, to develop best-in-class qPCR reagents. Furthermore, we did a comprehensive evaluation of commercially-available qPCR and RT-qPCR reagents, and developed a method of analysis that allows you to quickly compare and evaluate the performance of these products. We wanted to be sure that Luna products will perform to your expectations for all your targets.

Luna products offer exceptional sensitivity, reproducibility and qPCR performance



qPCR targeting human GAPDH was performed using the Luna Universal Probe qPCR Master Mix over a 6-log range of input template concentrations (20 ng – 0.2 pg Jurkat-derived cDNA) with 8 replicates at each concentration. cDNA was generated from Jurkat total RNA using the NEB Protoscript® II First Strand cDNA Synthesis Kit (NEB #E6560). NTC = non-template control

Evaluation of commercially-available dye-based qPCR reagents demonstrates the robustness and specificity of Luna

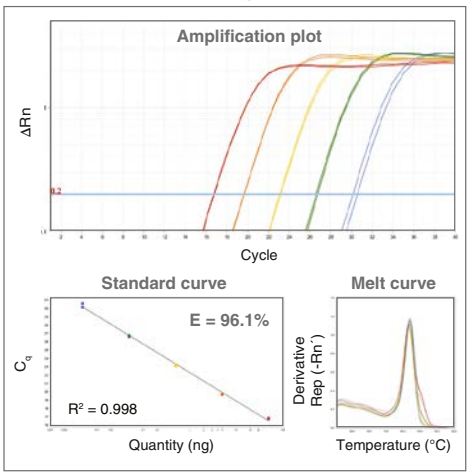


qPCR reagents from NEB and other manufacturers were tested across 16–18 qPCR targets varying in abundance, length and %GC, using either Jurkat genomic DNA or Jurkat-derived cDNA as input (10 genomic DNA targets and 8 cDNA targets on a Bio-Rad real-time instrument, 9 genomic and 7 cDNA targets on an ABI instrument). For each testing condition, data was collected by 2 users and according to manufacturer's specifications. Results were evaluated for efficiency, low input detection and lack of non-template amplification (where ΔC_q = average C_q of lowest input – average C_q of non-template control). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score). Bar graph indicates % of targets that met acceptable performance criteria (indicated by green box on dot plot and Quality Score > 3). Results for NEB and other major manufacturers are shown: Bio-Rad, SsoAdvanced™ Universal SYBR® Green Supermix; Roche®, FastStart™ SYBR Green Master; QIAGEN, QuantiTect® SYBR Green PCR Kit; ABI, PowerUP® SYBR Green Master Mix; Promega, GoTaq® qPCR Master Mix. NEB's Luna Universal qPCR Master Mix outperformed all other reagents tested.

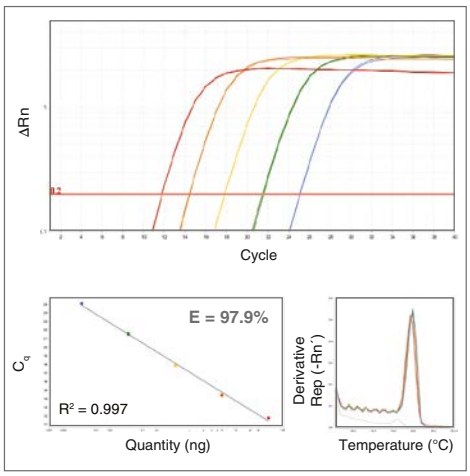
Your qPCR & RT-qPCR

Luna products provide sensitive, accurate detection & quantitation across a wide variety of genomic DNA sources

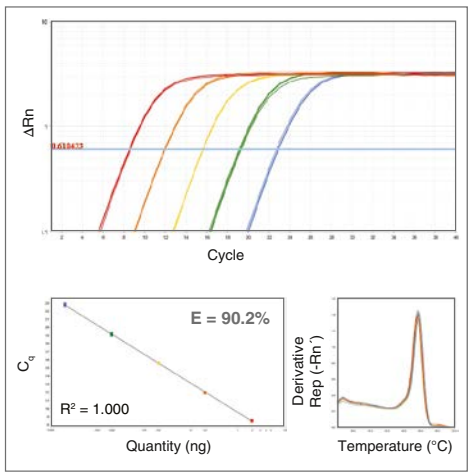
Mouse kidney – β-actin



Tobacco leaf – PsbB



Yeast – 18S



qPCR targets were quantitated with 50 ng – 0.5 pg genomic DNA as input using an ABI 7500 Fast real-time instrument. Genomic DNA was purified by typical column-based methods. In these examples, strong performance can be observed in the amplification of ACTB (encoding β-actin) from Mouse kidney genomic DNA, psbB (Photosystem II CP47 reaction center protein PsbB) from Tobacco, and RDN18 (18S ribosomal RNA) from Yeast.

Probe- versus Dye-based Detection Methods

Which should I choose for my qPCR?

qPCR is typically measured in one of two ways: either an intercalating dye that fluoresces more strongly upon binding to double-stranded DNA, or a fluorescently-labeled "probe" oligonucleotide that anneals to a specific sequence in the PCR amplicon.

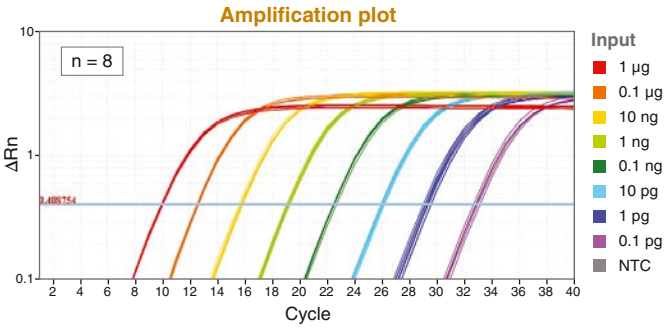
Dye-based detection requires only the addition of PCR primers, making it a cost-effective qPCR option. However, the intercalating dye will detect any dsDNA produced in the reaction. Therefore, off-target and non-template amplification (NTC) can be observed for some primer sets, resulting in inaccurate quantitation. Denaturation (melt) curves performed after the PCR can be used to distinguish between correct and nonspecific products. Additionally, only a single amplicon can be measured in a dye-based qPCR with no ability to perform multiplex reactions.

Probe-based detection requires designing and obtaining a sequence-specific fluorescently-labeled probe oligonucleotide in addition to typical PCR primers. This increases assay costs, but probe-based qPCR experiments benefit from extreme specificity and are unlikely to result in inaccurate quantification due to NTC amplification. Multiplex reactions are possible with probes, as different amplicons can be designed with unique fluorophores according to the optical capabilities of the qPCR instrument.

Optimize Your One-Step RT-qPCR/RT-PCR with Unique WarmStart Technology

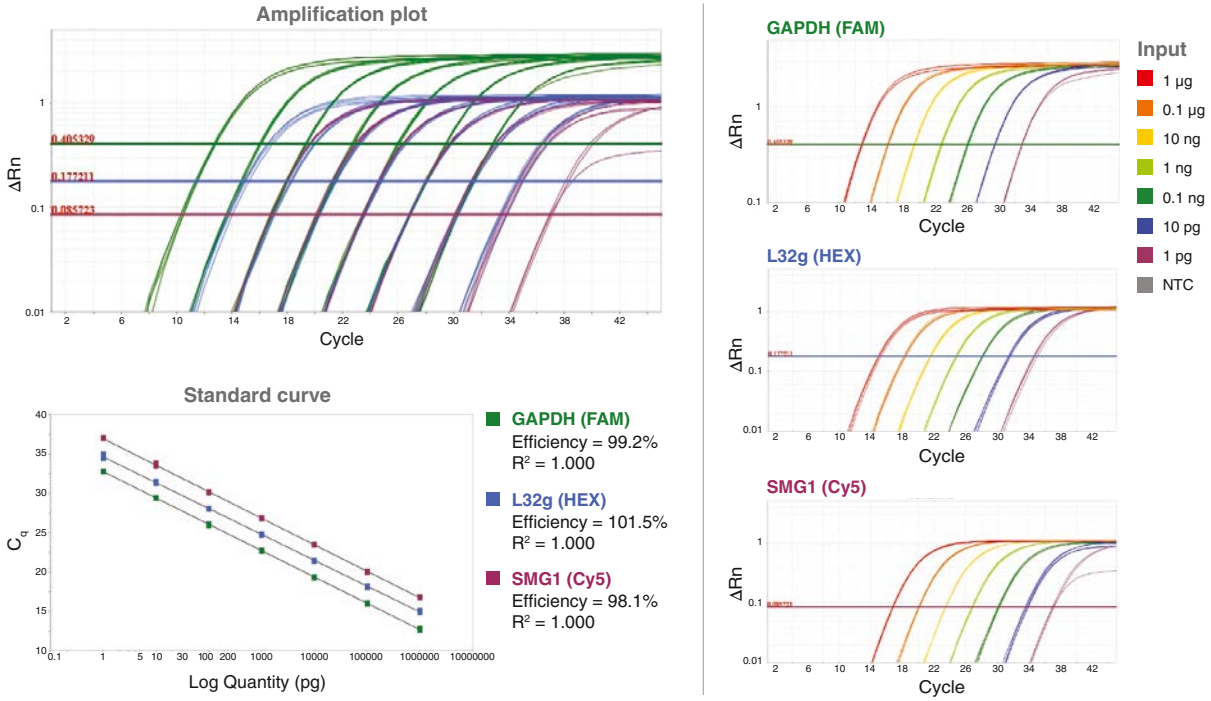
Luna and LunaScript products contain a novel, *in silico*-designed reverse transcriptase (RT) engineered for improved performance. One-Step RT-qPCR/RT-PCR products contain Luna WarmStart Reverse Transcriptase and Hot Start *Taq* DNA Polymerase, which utilize a temperature-sensitive, reversible aptamer, which inhibits activity below 45°C. This enables room-temperature reaction setup and prevents undesired non-specific activity. Furthermore, the Luna WarmStart RT has increased thermostability, improving performance at higher reaction temperatures.

Luna RT-qPCR products offer exceptional sensitivity, reproducibility and performance



RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit over an 8-log range of input template concentrations (1 µg – 0.1 pg Jurkat total RNA) with 8 replicates at each concentration. Reaction setup and cycling conditions followed recommended protocols, including a 10-minute RT step at 55°C for the thermostable Luna WarmStart Reverse Transcriptase. NTC = non-template control.

Luna RT-qPCR products offer robust performance in multiplex applications



Multiplex RT-qPCR targeting human GAPDH, ribosomal protein L32g and PI3-Kinase-Related Kinase SMG1 was performed using the Luna Universal Probe One-Step RT-qPCR Kit over a 7-log range of input template concentrations (1 µg – 1 pg Jurkat total RNA) with 4 replicates at each concentration. Amplification plots are shown both overlayed (left) and for each multiplex target (right). To account for copy number differences, 0.4 µM primer was used for lower-copy target (SMG1) and 0.2 µM primer for higher-copy targets (L32g and GAPDH). Luna maintains superior efficiency, reproducibility, sensitivity and performance in multiplex RT-qPCR. NTC = non-template control

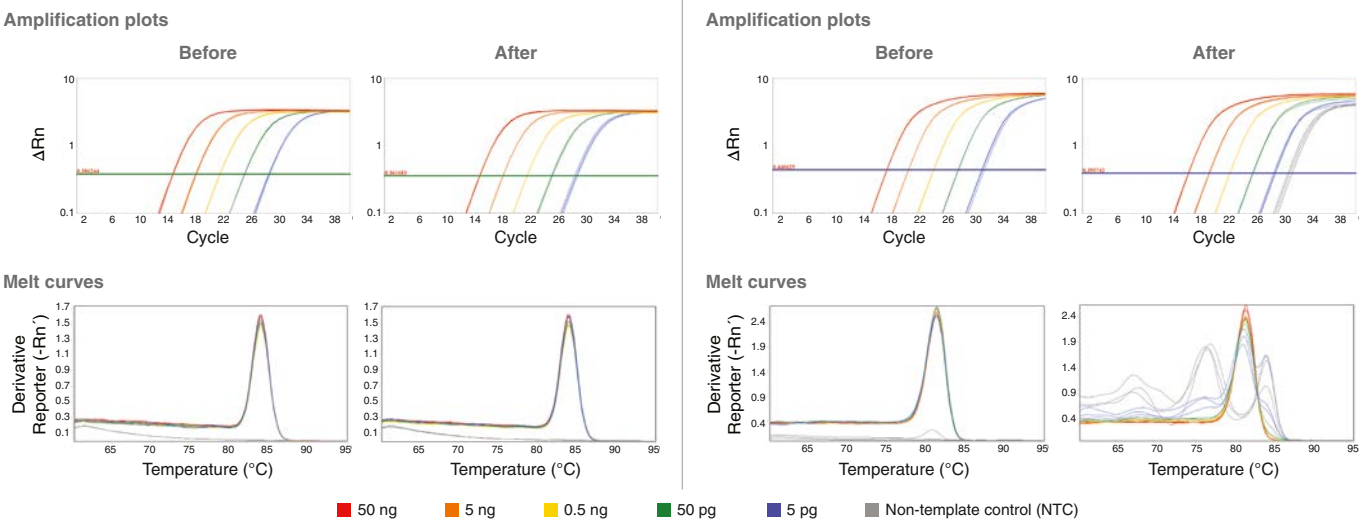
What is Luna WarmStart Reverse Transcriptase?

"WarmStart" is the term we use to describe a mesophilic enzyme that is inactive at room temperature, and becomes active when the reaction is warmed above approximately 40°C. This feature enables a flexible reaction setup and improves reaction specificity and thermostability.

Luna WarmStart Reverse Transcriptase prevents spurious amplification resulting from room-temperature pre-incubation

NEB Luna Universal One-Step RT-qPCR Kit
(featuring Luna WarmStart Reverse Transcriptase)

ABI Power SYBR® Green 1-Step Kit
(featuring ArrayScript™ UP Reverse Transcriptase)

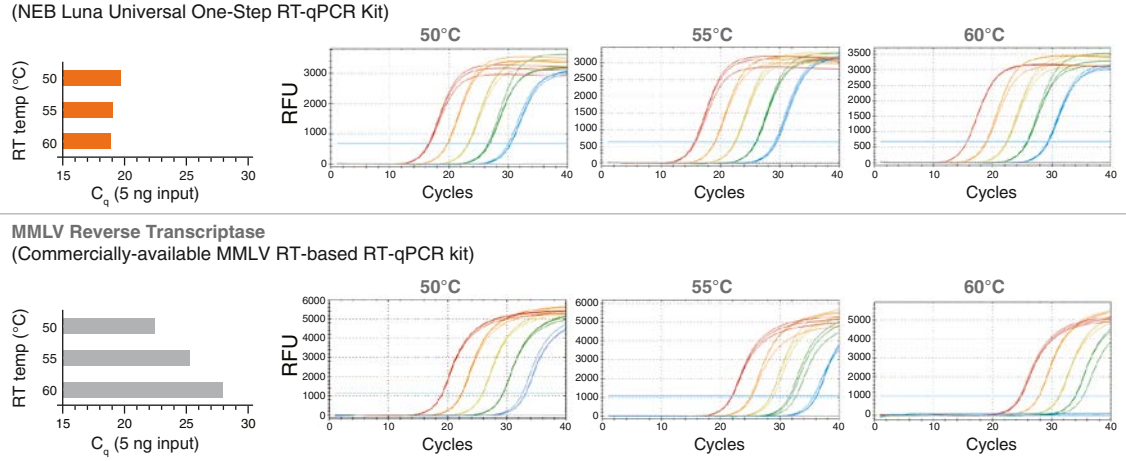


RT-qPCR targeting human ribosomal protein L32 was performed before and after a 24-hour incubation at room temperature, with triplicate reactions for a 5-log range of input human (Jurkat) total RNA and a non-template control. The Luna Universal One-Step RT-qPCR Kit featuring Luna WarmStart Reverse Transcriptase exhibited robust performance and no detectable non-template amplification, either with or without a 24 hour, 25°C pre-incubation, while the ABI 1-Step Kit, featuring a non-WarmStart reverse transcriptase, exhibited significant non-template amplification.

The increased thermostability of Luna WarmStart Reverse Transcriptase improves performance at higher reaction temperatures

A **Luna WarmStart Reverse Transcriptase**
(NEB Luna Universal One-Step RT-qPCR Kit)

B **MMLV Reverse Transcriptase**
(Commercially-available MMLV RT-based RT-qPCR kit)



RT-qPCR experiments targeting human ribosomal protein L32 RNA were performed in triplicate over a 5-log range of input human (Jurkat) total RNA (5 pg – 50 ng) using an initial 10 min RT step performed at 50°C – 60°C, as indicated.

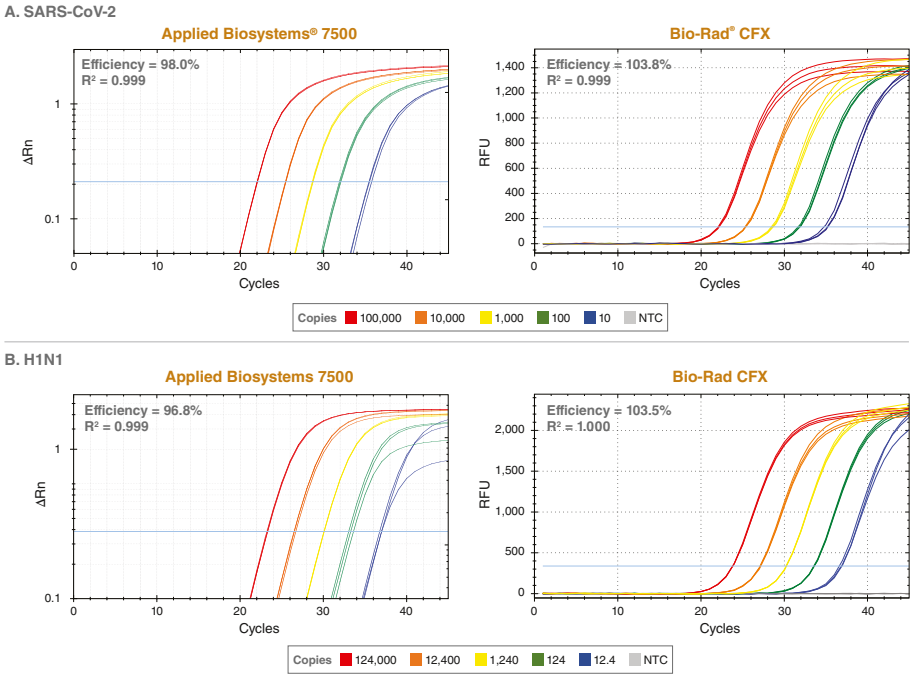
A. Luna WarmStart Reverse Transcriptase (recommended incubation temperature: 55°C) exhibited rapid C_q values (bar graph) and robust RT-qPCR performance (amplification plots) at each temperature, indicating that efficient reverse transcription was not perturbed by reaction temperature alterations.

B. In contrast, a commercially available MMLV (recommended incubation temperature: 50°C) exhibited delayed (increased) C_q values, poorer performance, and loss of low-input detection at elevated temperatures, consistent with loss of RT activity.

Optimized Viral RNA Detection

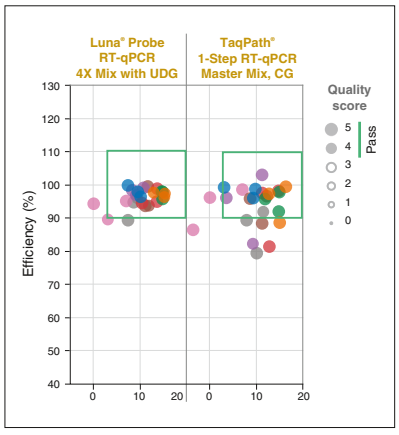
The Luna Probe One-Step RT-qPCR Mix with UDG (NEB #M3019) is supplied at a 4X concentration and enables higher amounts of sample input, which is relevant for applications where RNA present in low abundance is of interest, such as pathogen detection. Performance in multiplexing applications has been optimized, with sensitive, linear detection achieved for up to 5 targets across a range of inputs. The mix features Luna WarmStart RT, Hot Start *Taq* DNA Polymerase, dNTPs, a universal passive reference dye, and Murine RNase Inhibitor in an optimized buffer. The inclusion of dUTP and UDG in the master mix reduces the possibility of carryover contamination between reactions.

Robust amplification and detection of different viral RNA with Luna Probe One-Step RT-qPCR 4X Mix with UDG



RT-qPCR targeting SARS-CoV-2 (N1 target) and H. influenza H1N1 (HA target) was performed using the Luna Probe One-Step RT-qPCR Mix with UDG. Performance in 20 µl reactions was evaluated in two real-time instruments over a 5-log range of (A) 10–100,000 copies Synthetic SARS-CoV-2 RNA Control 2 (Twist Bioscience®, SKU: 102024) diluted in 10 ng of Jurkat total RNA (BioChain, #R1255815-50) and (B) 12.4–124,000 copies Influenza A (H1N1) RNA (ATCC® VR-95-DQ™) diluted in 10 ng Jurkat total RNA. Sensitive, linear performance can be observed in the amplification of both viral targets.

The Luna Probe One-Step RT-qPCR 4X Mix with UDG outperforms comparable products



One-step RT-qPCR was tested on 8 RT-qPCR targets (indicated by color) varying in abundance, length, and %GC. Data was collected on multiple days by two users according to manufacturer's recommendations using the Applied Biosystems® QuantStudio® 6 real-time PCR system. Results were evaluated for efficiency, low input detection and lack of non-template amplification (where ΔC_q = average C_q of non-template control – average C_q of lowest input). In addition, consistency, reproducibility and overall curve quality were assessed based on metrics described previously (Quality Score). Although both products performed reasonably well, NEB's Luna Universal Probe One-Step RT-qPCR Kit outperformed the TaqPath® 1-Step RT-qPCR Master Mix, CG, as evidenced by the higher number of experiments whose results fell in the green box.

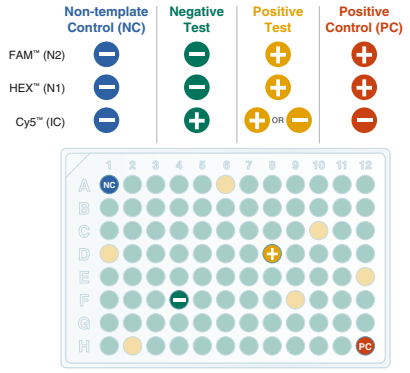
Luna Probe RT-qPCR 4X Mix with UDG (NEB #M3019)

Number of tubes	1
Concentration (sample-limiting)	4X
dUTP included	✓
UDG included	✓
Universal ROX included	✓

Optimized SARS-CoV-2 RNA Detection

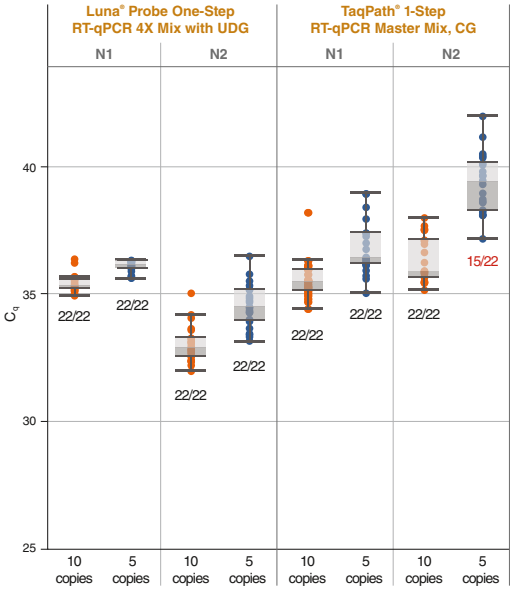
The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit (NEB #E3019) is a real-time RT-PCR assay for the qualitative detection of SARS-CoV-2 nucleic acid. The primer/probe mix is specific to two regions of the SARS-CoV-2 virus N gene [based on sequences provided by the Centers for Disease Control and Prevention (CDC)]. The probes have been modified to contain different fluorophores (N1: HEX; N2: FAM) to enable multiplexing. To ensure the integrity of the input material and absence of inhibition, an internal control primer and probe set, designed to amplify the human RNase P gene, is also included in the primer mix. The kit also includes the Luna Probe One-Step RT-qPCR Mix with UDG (NEB #M3019) and a positive control containing the full SARS-CoV-2 nucleocapsid protein (N gene).

Example Assay Setup and Anticipated Results



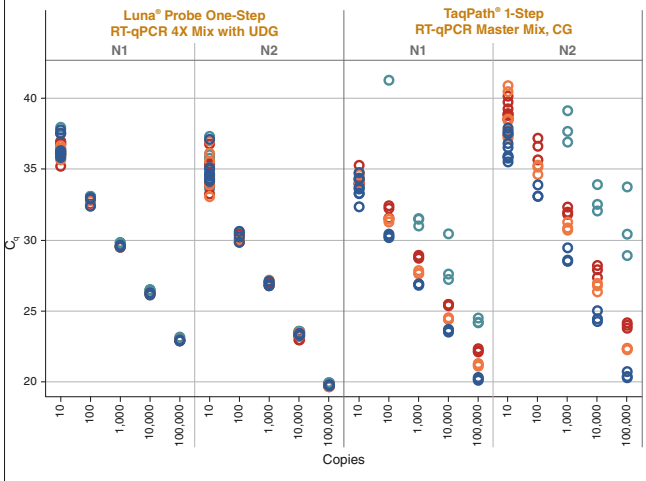
Using the Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit, up to 94 different samples can be assessed in a single 96-well plate. Anticipated results for each sample type are shown (in each fluorophore channel).

The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit demonstrates a lower limit of detection than TaqPath® 1-Step RT-qPCR Master Mix, CG



LOD comparison using: Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit for multiplex RT-qPCR targeting 2019-nCoV_N1 target (HEX) and 2019-nCoV_N2 target (FAM), according to reaction and cycling conditions provided in the E3019 product manual, and TaqPath 1-Step RT-qPCR Master Mix, CG for singleplex RT-qPCR targeting 2019-nCoV_N1 (FAM) and 2019-nCoV_N2 (FAM), according to the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel guidelines. Performance was evaluated using Synthetic Twist SARS-CoV-2 RNA Control 2 diluted in 10 ng of Jurkat total RNA. Data was collected on an Applied Biosystems 7500 Fast real-time instrument (96-well, 20 µl reactions). Under these conditions, the Luna Kit has an LOD of 5 copies/reaction for both targets while the LOD using TaqPath is 10 copies/reaction for these targets.

Room temperature stability of Luna RT-qPCR Mix enables workflow flexibility



C_q delay

	After 2 hrs		After 5 hrs		After 24 hrs	
	N1	N2	N1	N2	N1	N2
Luna (SP)	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Luna (MP)	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
TaqPath (SP)	1.0	1.9	1.8	3.1	>6	>9

Singleplex (SP) and multiplex (MP) RT-qPCR targeting 2019-nCoV_N1 (HEX) and 2019-nCoV_N2 (FAM) was performed using the Luna Probe One-Step RT-qPCR 4X Mix with UDG, according to reaction and cycling conditions provided in the NEB #E3019 product manual. Singleplex RT-qPCR targeting 2019-nCoV_N1 (FAM) and 2019-nCoV_N2 (FAM) was performed using TaqPath 1-Step RT-qPCR Master Mix, CG, according to the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel guidelines. Performance was evaluated over a 5-log range (100,000-10 copies) of Synthetic SARS-CoV-2 RNA Control 2 diluted in 10 ng of Jurkat total RNA. RT-qPCR reactions were incubated at room temperature for 0, 2, 5 and 24 hours before running on an Applied Biosystems 7500 Fast real-time instrument (96-well, 20 µl reactions). Using synthetic Twist RNA, consistent performance is observed with up to 24 hours of room temperature incubation with the Luna probe One-Step RT-qPCR Mix, while TaqPath showed a C_q delay ≥1 at 2 hours with declining performance as incubation time increased.

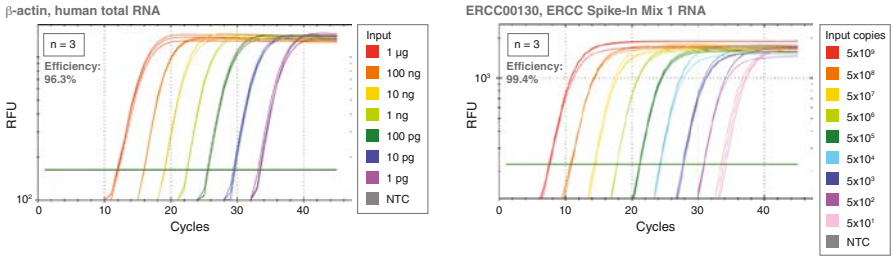
Speed up Your Two-Step RT-qPCR: LunaScript RT SuperMix Kit

Two-step RT-qPCR uncouples cDNA synthesis and qPCR analysis, allowing greater freedom in selecting reverse transcriptases and qPCR reagents separately. This flexibility can be useful for controlling sequence representation, qPCR efficiency, and optimization of reaction conditions when working with difficult RT-qPCR reactions or low RNA inputs.

The LunaScript RT SuperMix Kit (NEB #E3010) is optimized for first-strand cDNA synthesis in the context of a two-step RT-qPCR workflow. It employs the Luna Reverse Transcriptase in a convenient supermix format containing random hexamer and oligo-dT primers, dNTPs, and Murine RNase Inhibitor. This kit delivers best-in-class performance and requires the shortest reaction time (< 15 min) and tolerates elevated temperatures (55°–65°C) for working with difficult templates.

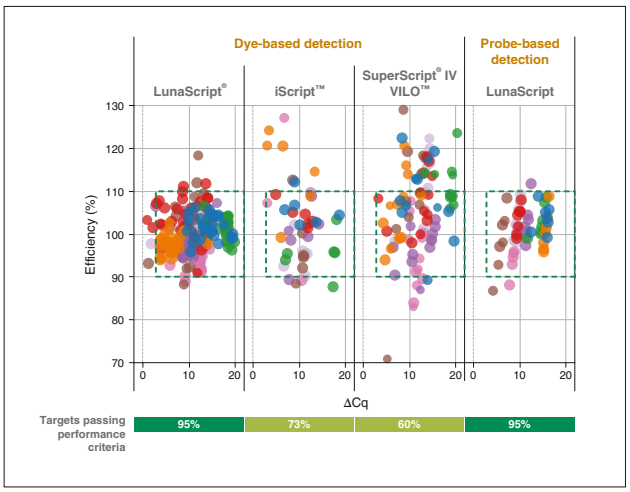
The cDNA products generated by LunaScript have been extensively evaluated in qPCR using the Luna qPCR Master Mixes (NEB #M3003/M3004). In combination, these products provide a two-step RT-qPCR workflow with excellent sensitivity and accurate, linear quantitations.

The LunaScript RT SuperMix Kit offers exceptional sensitivity, linearity and reproducibility in two-step RT-qPCR workflows



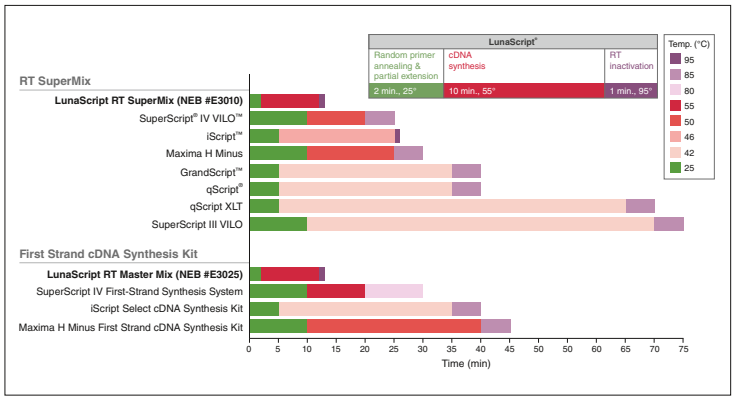
RNA was converted to cDNA using the 1X LunaScript RT SuperMix in 20 µl reactions using standard reaction conditions (25°C/2 min, 55°C/10 min, 95°C/1 min). cDNA was then quantitated by qPCR using the Luna Universal qPCR Master Mix (NEB #M3003) and 1 µl of cDNA product as template, with triplicate reactions at each input concentration. A. A serial dilution of Jurkat total RNA (1 µg–1 pg) was converted to cDNA and then quantitated by qPCR using a β-actin target. B. ERCC (External RNA Controls Consortium) mix1 RNA containing 5 x 10^9 to 50 copies of ERCC00130 (~10 ng–10 fg) was converted to cDNA and then quantitated by qPCR.

The LunaScript RT SuperMix Kit demonstrates superior linear detection of RNA targets



Commercially available cDNA supermixes were used according to manufacturer's recommendations to generate cDNA from 1 µg–100 pg human (Jurkat) total RNA. cDNA products were then evaluated by qPCR using eight targets varying in abundance, length and %GC. qPCR detection was performed using the Luna Universal qPCR Master Mix (NEB #M3003) or Luna Universal Probe qPCR Master Mix (NEB #M3004). Results were evaluated for efficiency and ΔC_q, where ΔC_q measures low input detection and lack of non-template control (NTC) amplification (ΔC_q = average C_q of NTC - average C_q of lowest input). Green box indicates target performance criteria (Efficiency = 90-110%, $\Delta C_q \geq 3$).

At just 13 minutes, the LunaScript RT SuperMix Kit offers the shortest available first-strand cDNA synthesis protocol



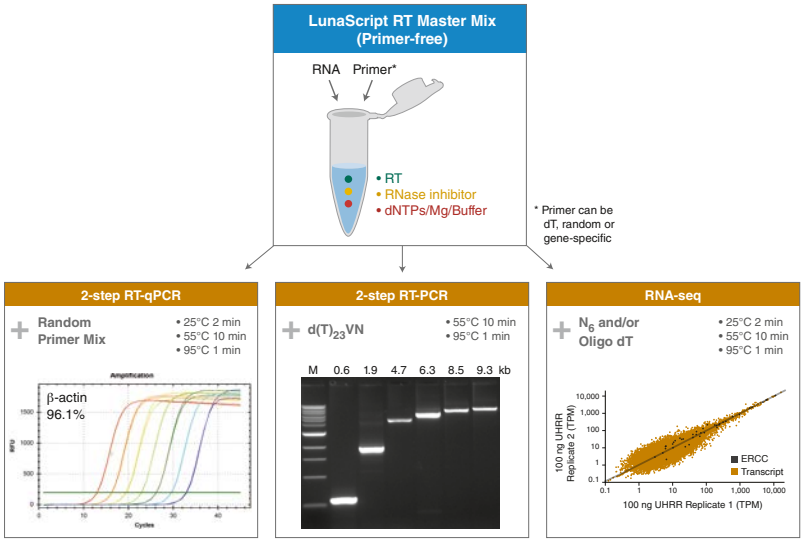
Comparison of recommended protocols for cDNA synthesis. Both LunaScript RT SuperMix Kit and LunaScript RT Master Mix Kit require the shortest reaction time and tolerate elevated temperatures, reducing complications from RNA secondary structure.

Request a sample at
LUNAqPCR.com

Flexible cDNA Synthesis

The LunaScript RT Master Mix Kit (Primer-free) (NEB #E3025) features an optimized 5X master mix containing all the necessary components for first-strand cDNA synthesis, except primers. The mix includes the thermostable Luna Reverse Transcriptase, which supports cDNA synthesis at elevated temperatures, dNTPs, and Murine RNase Inhibitor to protect template RNA from degradation. The mix is compatible with random primers, oligo dT primers, and gene-specific primers, enabling maximum cDNA synthesis flexibility.

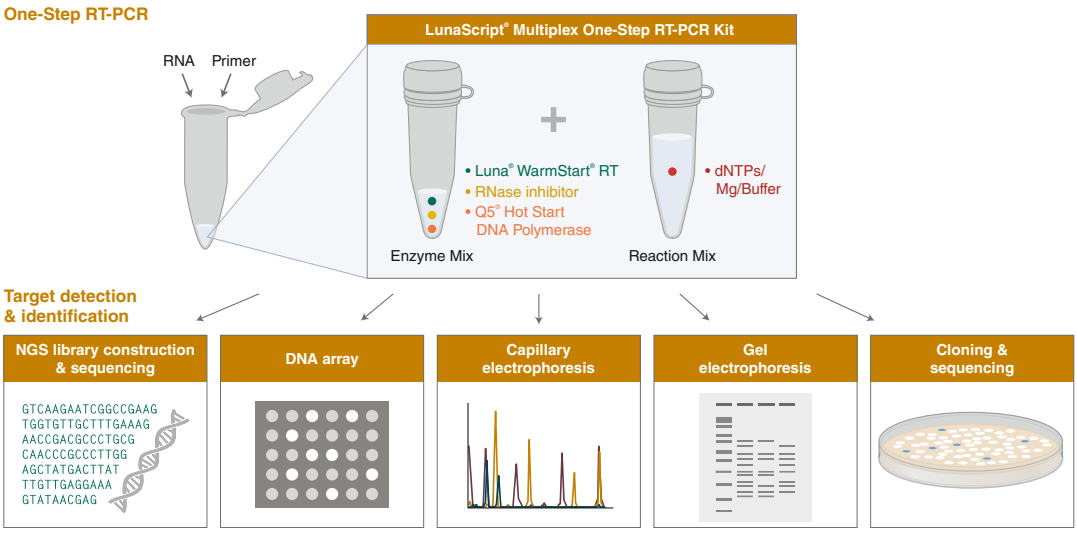
LunaScript RT Master Mix (Primer-free) supports numerous applications



By adding different primers including a Random Primer Mix, $d(T)_{23}VN$ oligos, or random hexamers, the LunaScript RT Master Mix can produce cDNA that is ideally suited for downstream applications such as RT-qPCR, RT-PCR, and RNA-seq studies.

Superior Multiplexing with Luna and Q5

The LunaScript Multiplex One-Step RT-PCR Kit (NEB #E1555) offers a streamlined protocol for cDNA synthesis and PCR amplification in a single reaction. It features Luna WarmStart RT and Q5 Hot Start High-Fidelity DNA Polymerase. The kit has robust multiplex target amplification capacity and enables various applications such as diagnostics, pathogen detection, and viral genome sequencing (including the ~50 amplicons per reaction used in ARTIC SARS-CoV-2 sequencing protocols).



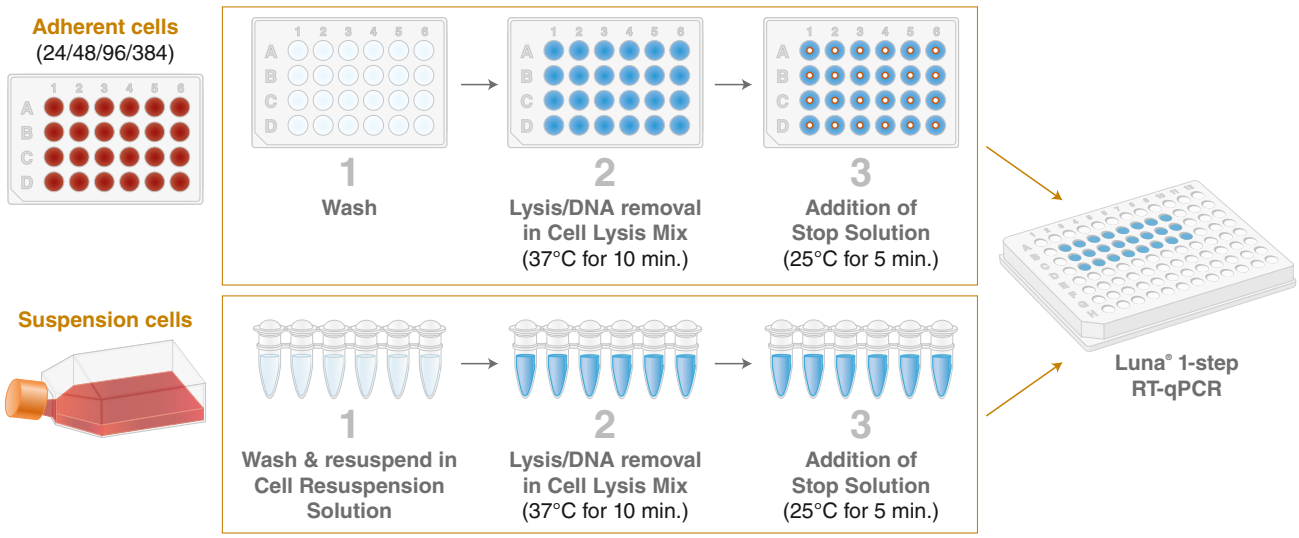
Go Direct to RNA Quantitation Without Purification: Luna Cell Ready Module and Kits

The Luna Cell Ready One-Step RT-qPCR Kit provides all the necessary components for direct RNA detection and quantitation from cultured mammalian and insect cell lines. Removing the need for traditional RNA extraction and purification, it offers a robust, sensitive, and convenient workflow for evaluating RNA expression levels in a 15-minute sample preparation protocol (prior to RT-qPCR).

The Luna Cell Ready Lysis One-Step RT-qPCR Kit is available for both dye (NEB #E3030) and probe (NEB #E3031) detection methods. In addition, the lysis module can be purchased separately (NEB #E3032).

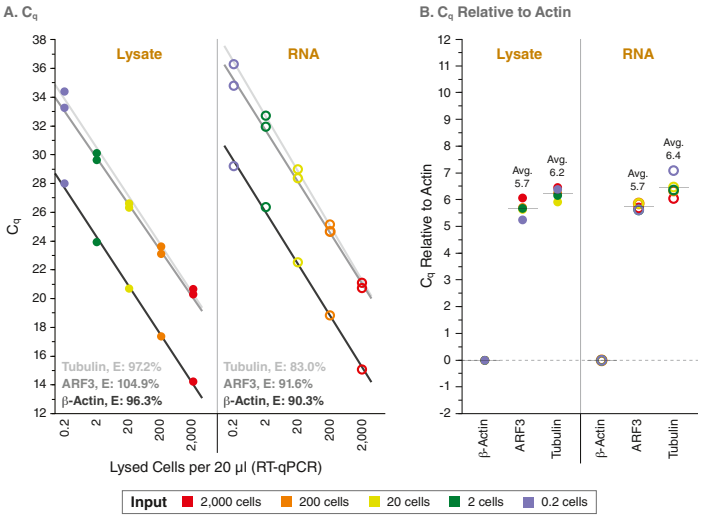
- Sensitive qPCR quantitation: linear RNA detection across a 5-log range of cell input dilutions
- Coordinated cell lysis, RNA release, and genomic DNA removal in a fast 15-minute protocol
- Increased convenience and minimal sample loss compared to alternative RNA purification methods
- Efficient cell lysate preparation from 10 to 100,000 cells across numerous cell lines
- Obtain reliable and precise results comparable to purified RNA
- Non-interfering, visible tracking dye eliminates pipetting errors
- Features Luna Universal One-Step RT-qPCR Kits (NEB #E3005/#E3006) for robust performance

Luna Cell Ready Workflow



The Luna Cell Ready One-Step RT-qPCR Kit provides all the necessary components for direct RNA detection and quantitation from cultured cells (up to 100,000 cells per 50 µl lysis reaction). Coordinating the actions of DNase I and the Luna Cell Ready Protease, the Luna Cell Ready Lysis Module offers a simple workflow resulting in effective cell lysis, RNA release, and genomic DNA removal simultaneously in a 15-minute protocol. Up to 2 µl lysate (equivalent to RNA from 0.2 - 4,000 cells) can be transferred into 20 µl downstream RT-qPCR reactions.

The Luna Cell Ready One-Step RT-qPCR kit offers reliable and precise RNA quantitation comparable to purified RNA across 5-log cell input.

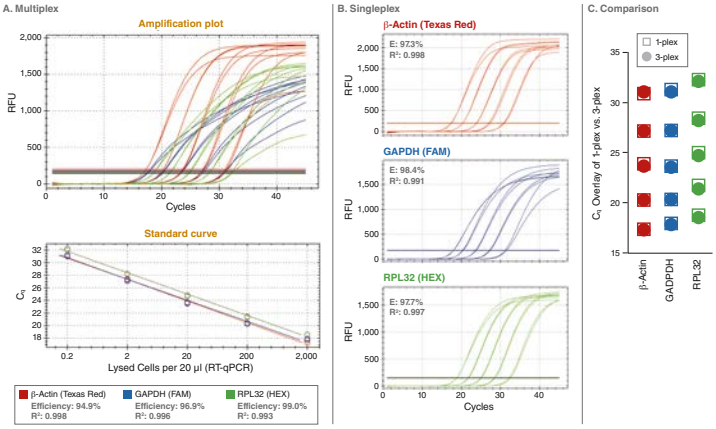


Serial dilutions of A549 cells (100,000–10) were lysed in 50 µl Luna Cell Ready lysis reactions (NEB #E3032) using standard reaction conditions (10 min lysis at 37°C, 5 min inactivation at 25°C). Alternatively, RNA was purified using a column-based RNA extraction kit.

A. Genes of interest (GOI) were then quantitated using the Luna Universal One-Step RT-qPCR Kit (NEB #E3005) with 1 µl of cell lysate (closed circles) or purified RNA (empty circles) as input (equal to 0.2–2,000 cells in a 20 µl RT-qPCR reaction), with duplicate reactions at each input concentration. Left: Detection of β-actin, an abundant target, and ARF3 and tubulin, two less abundant targets, across 5-logs of cell inputs. Efficiency (E) for each target is shown at the lower left corners of the panel.

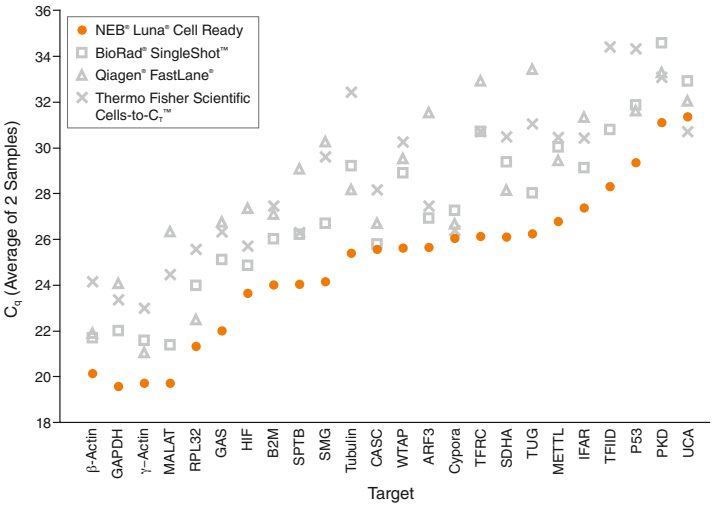
B. Cq relative to β-actin at each dilution (ΔCq (GOI)) was calculated from the data in (A) across 5-log cell inputs. The average Cqs are shown as bars.

The Luna Cell Ready Probe One-Step RT-qPCR kit offers sensitive and accurate quantitation of RNA directly from cell lysates across 5-log cell inputs.



Serial dilutions of HeLa cells (100,000–10) were lysed in 50 µl Luna Cell Ready lysis reactions (NEB #E3032) using standard reaction conditions (10 min lysis at 37°C, 5 min inactivation at 25°C). Genes of interest were then quantitated using the Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006) using 1 µl of cell lysate as input (equal to 0.2–2,000 cells in a 20 µl RT-qPCR reaction), with duplicate reactions at each input concentration. Results for β-actin (Texas Red), GAPDH (FAM), two abundant targets and RPL32 (HEX), a less abundant target, in multiplex (A), and singleplex reactions (B) are shown. Efficiency (E) and linearity (R2) are shown for each experiment. (C) Cq overlay of multiplex and singleplex of all 3 targets demonstrates compatibility of these results.

The Luna Cell Ready One-Step RT-qPCR Kit outperforms commercially available cell lysate One-Step RT-qPCR Kits with the earliest Cq on a large detection panel (23/24).



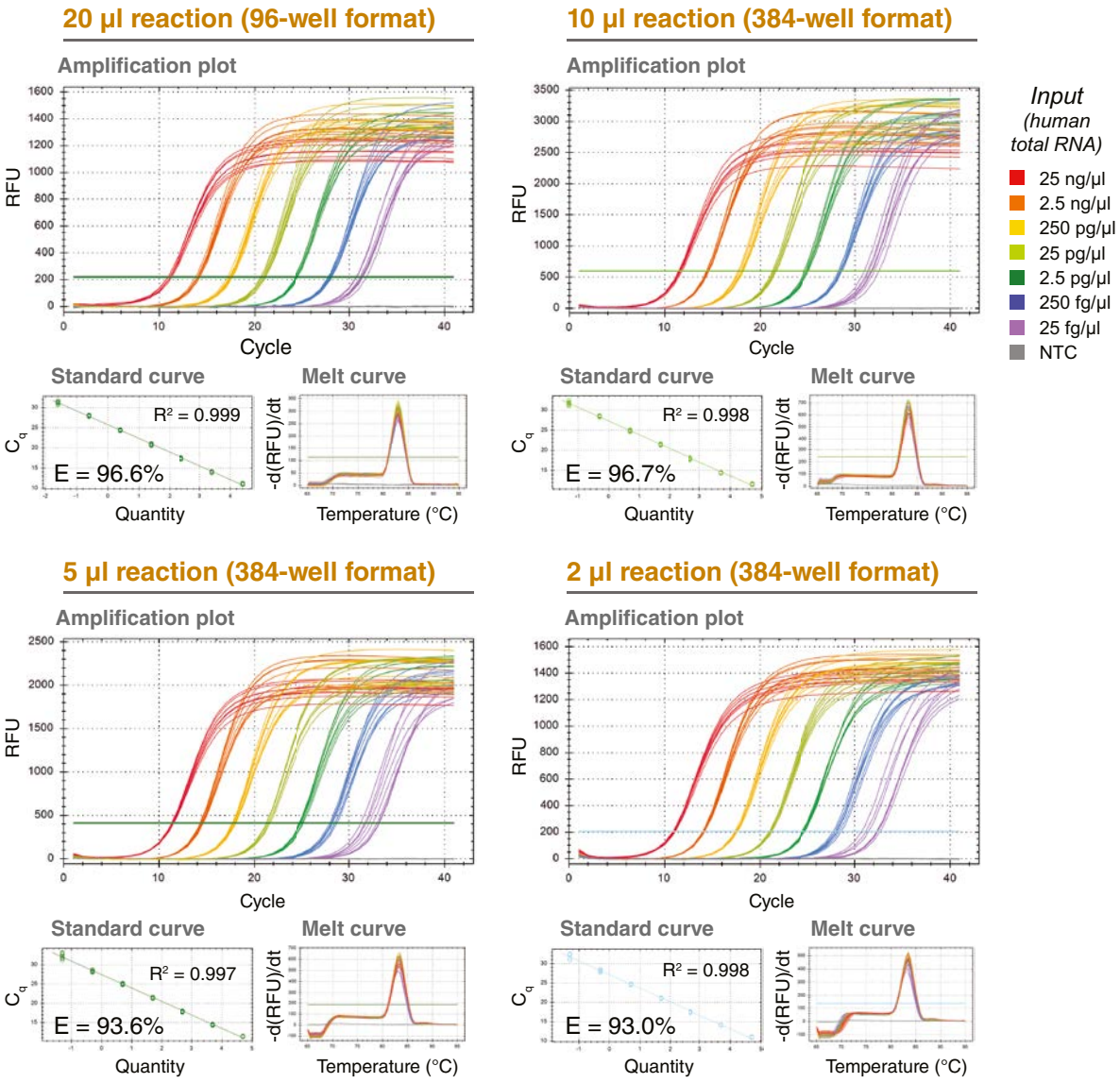
Approximately 2,500 A549 cells were lysed in 50 µl Luna Cell Ready lysis reactions (NEB #E3032) using standard reaction conditions or with commercially available kits following manufacturer-recommended protocols. Two biological replicates were processed for each kit. 24 genes of interest were then quantitated using the One-Step RT-qPCR module from each kit with 1 µl of cell lysate as input (equal to 50 cells in a 20 µl RT-qPCR reaction), with duplicate reactions for each biological sample. Average Cqs are shown for NEB (closed orange circles), BioRad (open squares), Qiagen (open triangles) and Thermo Fisher Scientific (crosses). To standardize results, 12% of total fluorescence was set as a threshold. The Luna Cell Ready One-Step RT-qPCR Kit shows the earliest Cq for 23/24 genes across variable expression levels, with an average of 2.3 Cq faster than BioRad, 3.8 Cq faster than Qiagen, and 3.6 Cq faster than Thermo Fisher Scientific.

Performance in High-throughput, Automated Workflows, & 384-Well Formats

The ability to conduct qPCR experiments in high-throughput formats offers many advantages: gene expression studies can be expanded to include more targets, multiple controls, and additional replicates; screens can be conducted at a larger scale; diagnostic tests can accommodate more patient samples. 384-well real-time PCR instruments are readily available, improving throughput over standard 96-well platforms, with automated liquid handling systems used to improve efficiency.

Luna qPCR/RT-qPCR reagents maintain their performance in high-throughput applications and are compatible with automated reaction setup systems. Sensitive detection and linear quantitation are maintained in 384-well formats at reduced reaction volumes.

Luna reagents are compatible with acoustic liquid transfer and reaction miniaturization.



The Beckman® Echo 525 coustic Liquid Handler was used to assemble Luna Universal One-Step RT-qPCR reactions at 10 µl, 5 µl and 2 µl volumes in a 384-well format, targeting human GAPDH for quantitation over a 7-log range of input template concentrations (25 ng/µl – 25 fg/µl Jurkat total RNA) with 8 replicates at each concentration. Linear quantitation and low-input detection were maintained in miniaturized reactions.

Ordering Information

PRODUCT NAME	NEB #	SIZE
Luna Universal qPCR Master Mix	M3003S/L/X/E	200/500/1,000/2,500 rxns
Luna Universal Probe qPCR Master Mix	M3004S/L/X/E	200/500/1,000/2,500 rxns
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/1,000/2,500 rxns
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/1,000/2,500 rxns
Luna Probe One-Step RT-qPCR Kit (No ROX)	E3007E	2,500 rxns
Luna Probe One-Step RT-qPCR 4X Mix with UDG	M3019S/L/X/E	200/500/1,000/2,000 rxns
Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit	E3019S/L	96/480 rxns
LunaScript RT Master Mix Kit (Primer-free)	E3025S/L	25/100 rxns
LunaScript RT SuperMix Kit	E3010S/L	25/100 rxns
LunaScript Multiplex One-Step RT-PCR Kit	E1555S/L	50/250 rxns
Luna Cell Ready One-Step RT-qPCR Kit	E3030S	100 rxns
Luna Cell Ready Probe One-Step RT-qPCR Kit	E3031S	100 rxns
Luna Cell Ready Lysis Module	E3032S	100 rxns (50 µl)
RELATED PRODUCTS		
Q5 High-Fidelity DNA Polymerase	M0491S/L	100/500 rxns
Hot Start *Taq* DNA Polymerase	M0495S/L	200/1,000 units
One*Taq* DNA Polymerase	M0480S/L/X	200/1,000/5,000 rxns
Bst DNA Polymerase, Large Fragment	M0275S/L/M	1,600/8,000/8,000 units
Bst DNA Polymerase,Full Length	M0328S	500 units
Bst 2.0 DNA Polymerase	M0537S/L/M	1,600/8,000/8,000 units
Bst 3.0 DNA Polymerase	M0374S/L/M	1,600/ 8,000/8,000 units
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1804S/L	100/500 reactions
WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)	M1708S/L	100/500 reactions
WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)	E1708S/L	100/500 reactions
ProtoScript II Reverse Transcriptase	M0368S/L/X	4,000/10,000/40,000 units
Exo-CIP Rapid PCR Cleanup Kit	E1050S/L	100/400 reactions

Request a sample at:
LUNAqPCR-com

Unparalleled confidence.

For over 25 years, New England Biolabs has been committed to the development of innovative, high quality tools for your PCR, qPCR and related amplification technologies. Our product quality, enzyme expertise and outstanding technical support bring unparalleled confidence to your experiments.

Featured Products Include:

- **Luna qPCR & RT-qPCR Reagents:** for rapid, sensitive and precise detection of RNA, DNA and cDNA targets
- **Q5 High-Fidelity DNA Polymerase:** for robust, ultra high-fidelity PCR
- **One*Taq*® DNA Polymerase:** for robust, routine PCR
- **ProtoScript® II Reverse Transcriptase:** for efficient reverse transcription
- ***Bst* DNA Polymerases:** for robust isothermal amplification
- **Exo-CIP™ Rapid PCR Cleanup Kit:** for rapid degradation of PCR primers and dephosphorylation of dNTPs following amplification
- **dNTPs:** ultrapure solution sets and mixes for a variety of applications

Learn more about NEB's portfolio or products for PCR, as well as qPCR, RT-qPCR, isothermal amplification and cDNA synthesis at **NEBPCR.com**.

GERMANY & AUSTRIA
New England Biolabs GmbH
Brüningstr. 50, Geb B852
65926 Frankfurt/Main, Germany

Tel: +49/(0)69/305-23140
Fax: +49/(0)69/305-23149

Free Call: 0800/246 5227 (Germany)
Free Call: 00800/246 52277 (Austria)
info.de@neb.com

www.neb-online.de

FRANCE
New England Biolabs France SAS
Genopole Campus 1, Bâtiment 6
5 rue Henri Desbruères
91030 Evry cedex, France

Tel.: 0800 100 632 (Customer Service)
Tel.: 0800 100 633 (Technical Service)

FAX.: 0800 100 610
info.fr@neb.com

www.neb-online.fr

HEADQUARTERS:
USA
New England Biolabs, Inc.

Telephone: (978) 927-5054
Toll Free (USA Orders): 1-800-632-5227
Toll Free (USA Tech): 1-800-632-7799

Fax: (978) 921-1350
info@neb.com
www.neb.com

www.neb.com

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices
Certified B Corporation

Featured Tools

Download the **NEB AR App** for iPhone® or iPad®. Scan the augmented reality butterfly icon located on the corner of the page to find videos, tutorials and immersive experiences.

LUNA – Version 5.0 – 9/21
x/f/yNEB126

Your local NEB distributor:

BeNeLux:
BIOKÉ
Tel: (+31) 71 720 0220
(BE: 0800 - 71640)
info@bioke.com
www.bioke.com

BULGARIA
ELTA 90M Ltd.
Tel: (+359) 2 983 96 49
Fax: (+359) 2983 2211, 9274 232
office@elta90.eu
www.elta90.com

CROATIA:
Diagnostica Skalpeli d.o.o.
Tel: (+385) 1 377 8484
Fax: (+385) 1 377 8585
skalpeli@skalpeli.hr
www.skalpeli.hr

CZECH REPUBLIC:
BioTech a.s.
Tel: (+420) 2727 017 39
info@ibiotech.cz
www.ibiotech.cz

DENMARK:
BioNordika Denmark A/S
Tel: (+45) 39 56 20 00
Fax: (+45) 39 56 19 42
info@bionordika.dk
www.bionordika.dk

FINLAND:
BioNordika Oy
Tel: (+358) 207 410 270
info@bionordika.fi
www.bionordika.fi

GREECE:
BIOLINE SCIENTIFIC
Tel: (+30) 210 522 6547
Fax: (+30) 210 524 4744
info@bioline.gr
www.bioline.gr

HUNGARY:
Kvalitex Kft.
Tel: (+361) 340 4700
Fax: (+361) 339 8274
info@kvalitex.hu
www.kvalitex.hu

ITALY:
EUROCLONE S.P.A.
Tel: (+39) 02 38 1951
info@euroclone.it
www.euroclonegroup.it

ISRAEL:
Ornat BioChemicals &
Lab Equipment
Tel: (+972) 8 947 7077
Fax: (+972) 8 936 3034
ornatbio@ornat.co.il
www.ornat.co.il

LITHUANIA:
Nanodiagnostika, Ltd.
Tel (+370) 525 052 44
lina@nanodiagnostika.lt
www.nanodiagnostika.lt

NORWAY:
BioNordika AS
Tel (+47) 2303 5800
info@bionordika.no
www.bionordika.no

POLAND:
Lab-JOT Ltd. Sp.z o.o. Sp.k.
Tel: (+48) 2233 598 84
Fax: (+48) 2233 598 19
biuro@labjot.com
www.labjot.com

PORTUGAL:
Werfen
Tel. (+351) 214 247 300
dac-pt@werfen.com
www.pt.werfen.com

ROMANIA:
BioZyme
Tel: (+40) 264 523 281
contact@biozyme.ro
www.biozyme.ro

RUSSIA and CIS:
SkyGen LLC
Tel: (+88) 0033 312 26
info@skygen.com
www.skygen.com

SERBIA:
Alfa Genetics d.o.o.
Tel: (+38) 1011 2084 252
office@alfagenetics.rs
www.alfagenetics.rs

SPAIN:
Werfen
Tel: (+34) 900 822 290
customerservice-es@werfen.com
www.es.werfen.com

SLOVAK REPUBLIC
BioTech s.r.o.
Tel: (+421) 254 7744 88
Fax: (+421) 232 2010 48
info@ibiotech.sk
www.ibiotech.sk

SLOVENIA:
Mikro+Polo d.o.o.
Tel: (+386) 2614 33 00
Fax: (+386) 2614 33 20
podpora@mikro-polo.si
www.mikro-polo.si

SOUTH AFRICA:
Inqaba Biotechnical Industries
(Pty) Ltd
Tel: (+27) 1234 358 29
info@inqababiotec.co.za
www.inqababiotec.co.za

SWEDEN, ESTONIA, LATVIA,
LITHUANIA:
BIONORDIKA SWEDEN AB
Tel: (+46) 830 60 10
info@bionordika.se
www.bionordika.se

SWITZERLAND:
BioConcept Ltd.
Tel: (+41) 6148 680 80
Fax: (+41) 6148 680 00
info@bioconcept.ch
www.bioconcept.ch

TURKEY:
EKA BIOLAB TEKNOLOJI
Tel. (+90) 312 248 13 24
Email: info@ekabiolab.com
www.ekabiolab.com

Distributors
For a complete list of all NEB subsidiaries and distributors, please go to:
www.neb.com/international-support