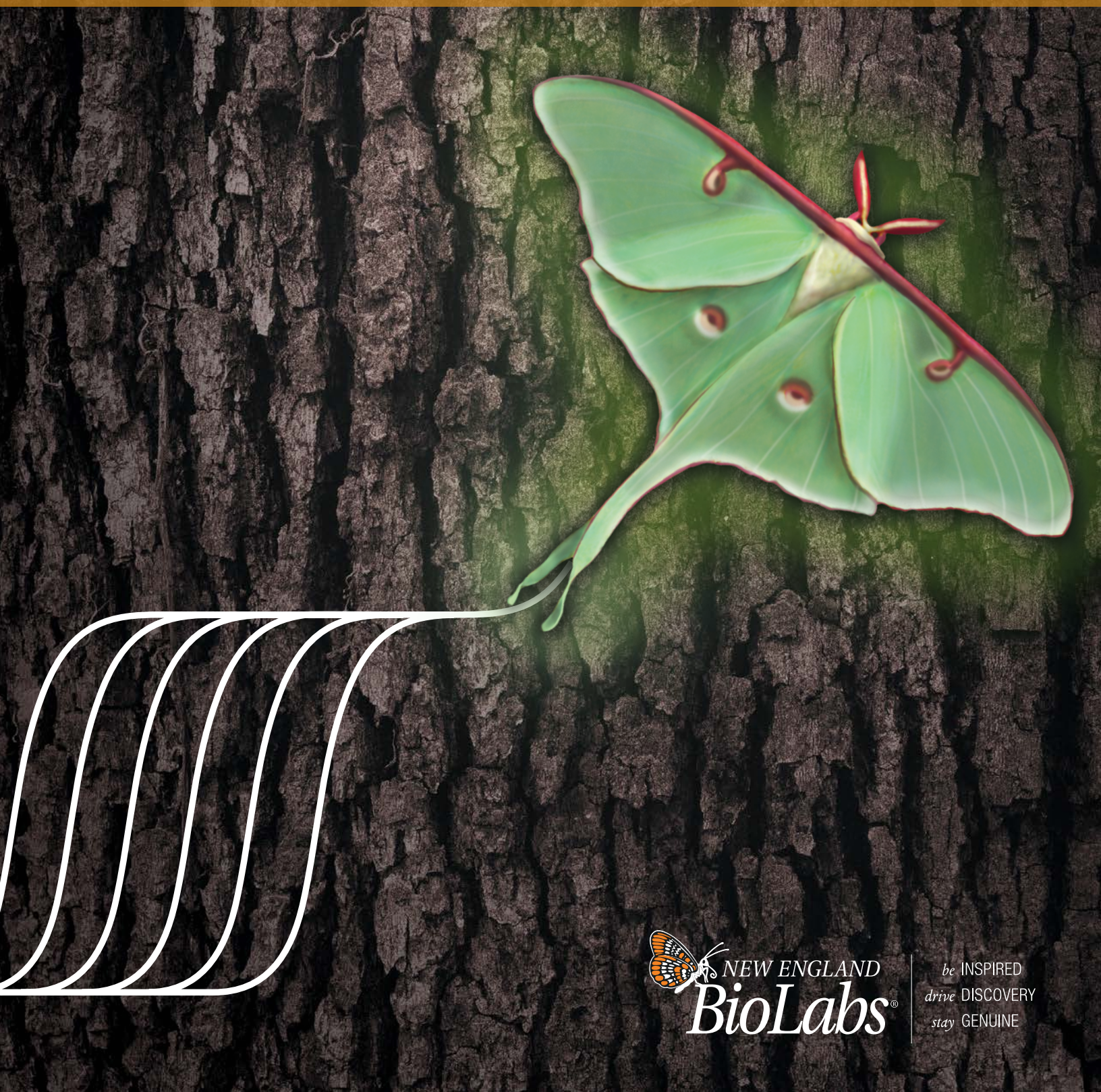
Now includes
Luna Cell Ready Module and Kits.

Luna® Universal qPCR & RT-qPCR

LIGHTING THE WAY™



NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE

Lighting the way

Luna products from NEB® are optimized for qPCR or RT-qPCR and are available for either intercalating dye or probe-based detection methods. All Luna products provide robust performance on diverse sample sources and target types.

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). A blue visible dye assists in tracking the reagents when pipetting into clear or white PCR plates.

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. Optimal results are obtained when paired with Luna Universal One-Step RT-qPCR kits.

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)
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

- One product per application simplifies selection
- Convenient master mix and supermix formats with user-friendly protocols simplify reaction setup
- Non-interfering, visible tracking dye eliminates pipetting errors

Optimize your One-Step RT-qPCR

- Luna Warmstart Reverse Transcriptase (RT) is a novel, thermostable RT with improved performance
- WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness
- Skip RNA purification and go direct from cells to RT-qPCR analysis with Luna Cell Ready Kits

Speed up your Two-Step RT-PCR

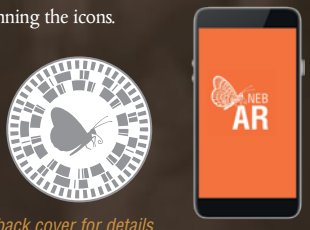
- LunaScript RT SuperMix Kit is validated for first strand cDNA synthesis with a fast 13-minute protocol
- Easily integrate into a two-step RT-qPCR workflow with Luna Universal qPCR Master Mixes

Learn more at LUNAqPCR.com

Explore and Discover

Download* the NEB Augmented Reality (AR) app and enjoy videos, tutorials and immersive experiences by scanning the icons.

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.

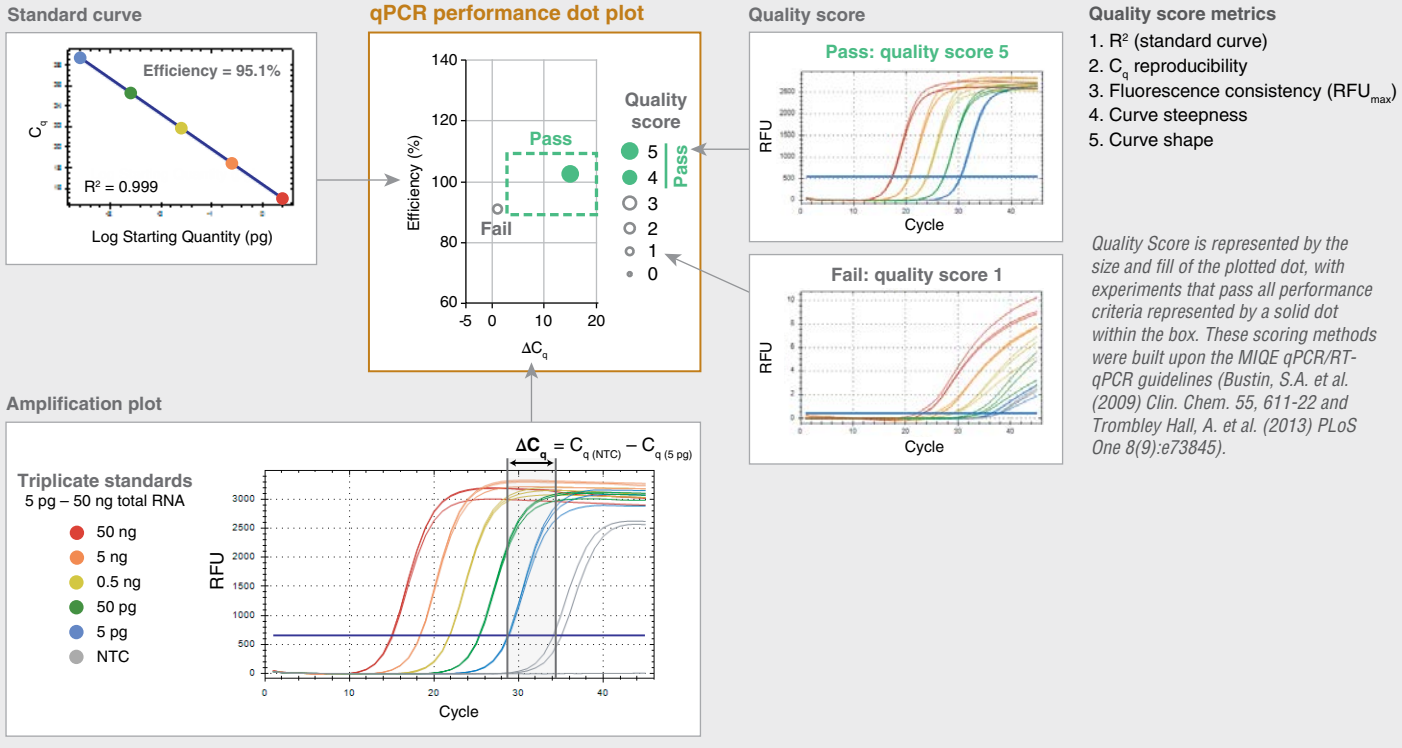
For each experiment, triplicate reactions are set up across a five-log range of input template concentrations (Amplification plot, bottom-left). Three non-template control (NTC) reactions are also included, for a total of 18 reactions per condition/target. Efficiency (%) is calculated (Standard plot, 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"



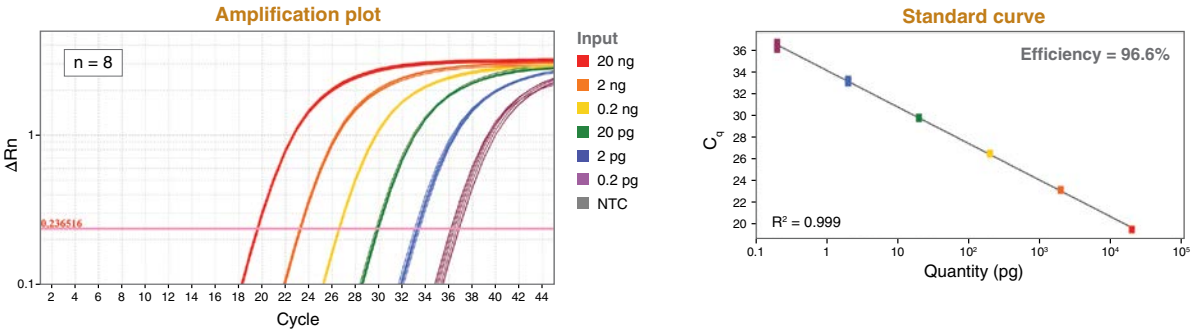
Quality Score is represented by the size and fill of the plotted dot, with experiments that pass all performance criteria represented by a solid dot within the box. These scoring methods were built upon the MIQE qPCR/RT-qPCR guidelines (Bustin, S.A. et al. (2009) Clin. Chem. 55, 611-22 and Trombley Hall,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

How can we ensure best in class performance with Luna?

Experience best-in-class performa

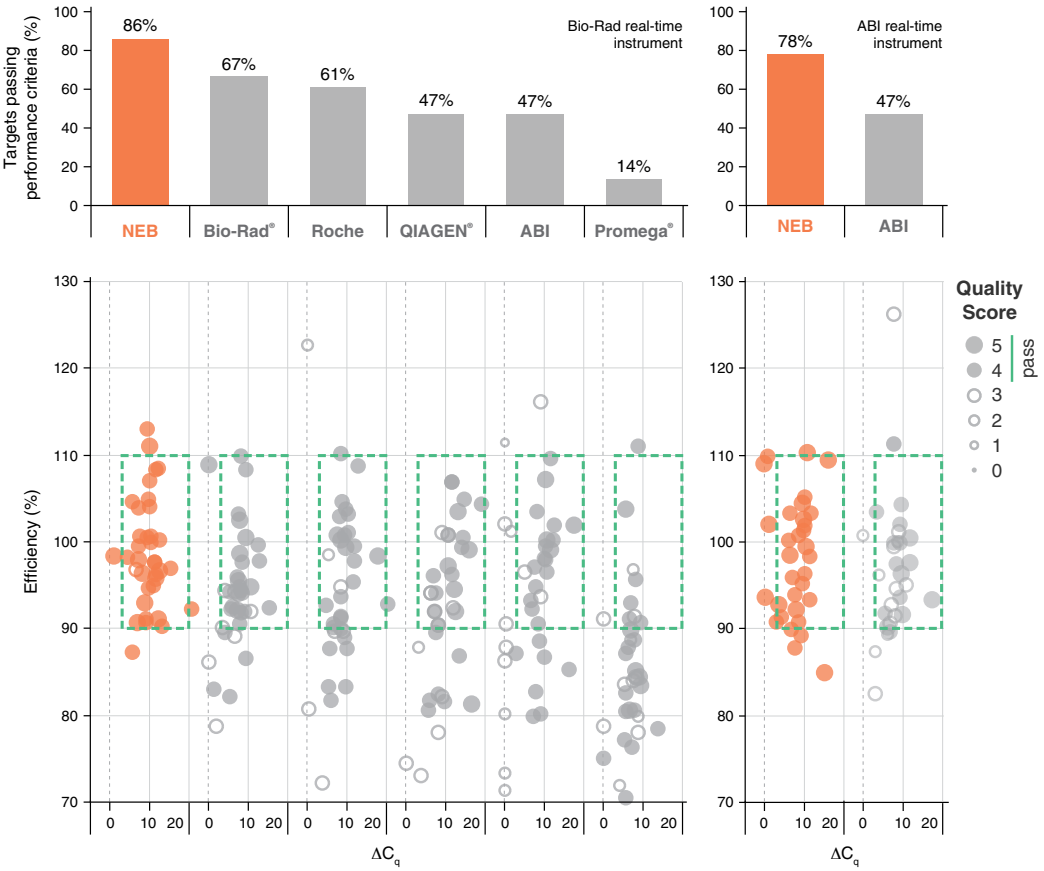
All NEB products undergo rigorous testing to ensure optimal performance, and Luna is no exception. We took into consideration numerous important traits when evaluating qPCR, including specificity, sensitivity, accuracy and reproducibility, to develop best-in-class qPCR reagents. Furthermore, we did a 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

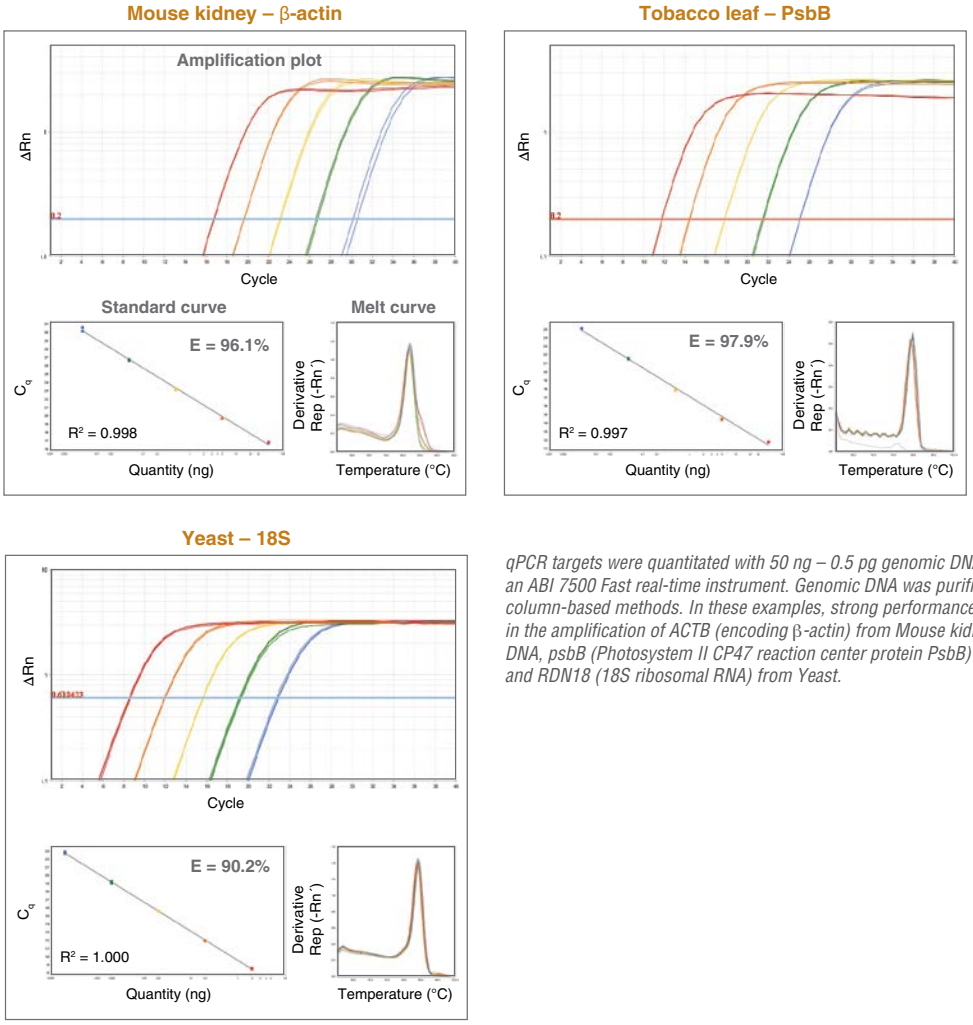
Evaluation of commercially-available dye-based qPCR reagents demonstrates the robustness and specificity of Luna



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.

nce for your qPCR & RT-qPCR

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



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.

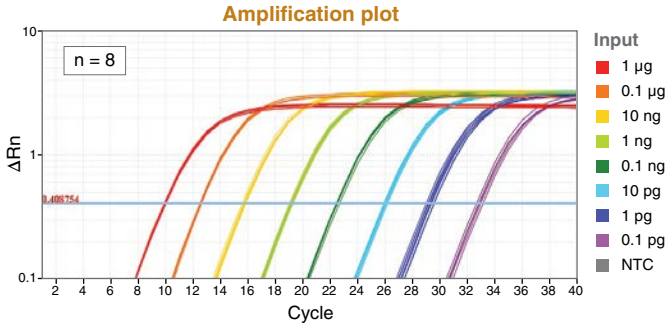
Dye-based detection requires only the addition of PCR primers, making it a cost-effective qPCR option. However, the intercalating dye will detect any dsDNA produced in the reaction. Therefore, off-target and non-template amplification (NTC) can be observed for some primer sets, resulting in inaccurate 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 with unique WarmStart technology

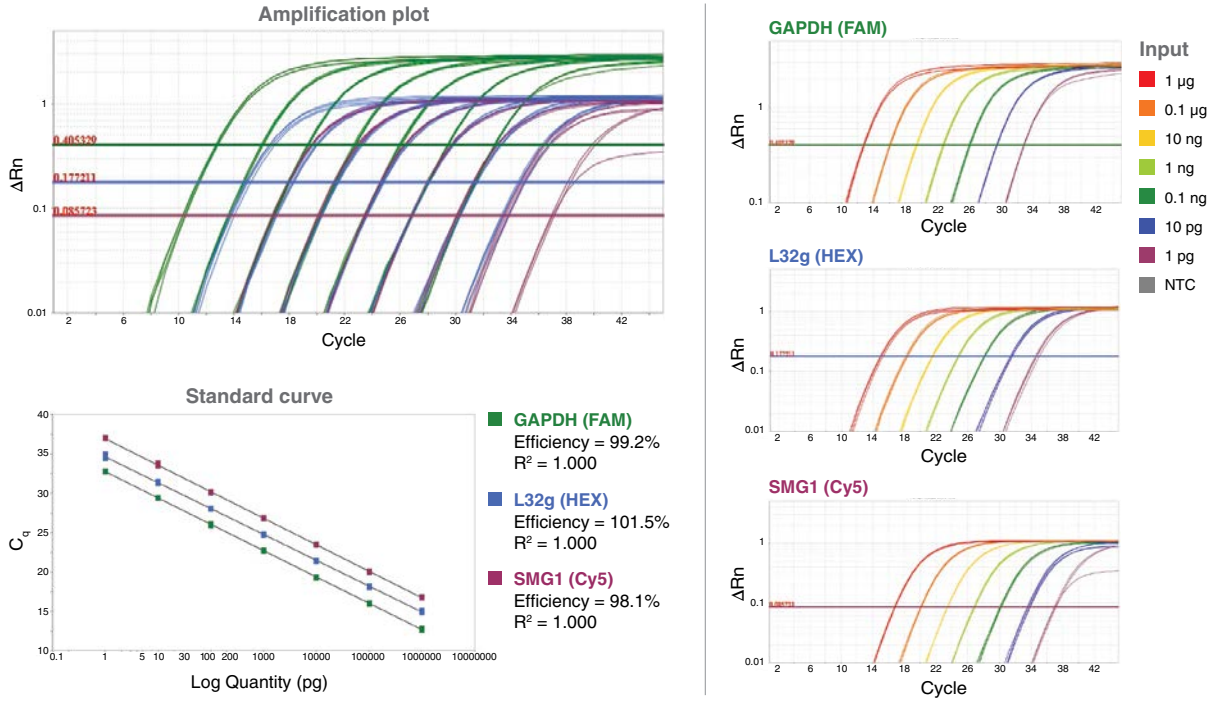
The Luna RT-qPCR kits contain a novel, *in silico*-designed reverse transcriptase (RT) engineered for improved performance. Both the Luna WarmStart Reverse Transcriptase and Hot Start *Taq* DNA Polymerase, included in these kits, utilize a temperature-sensitive, reversible aptamer, which inhibits activity below 45°C. This enables room temperature reaction setup and prevents undesired non-specific activity. Furthermore, the 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

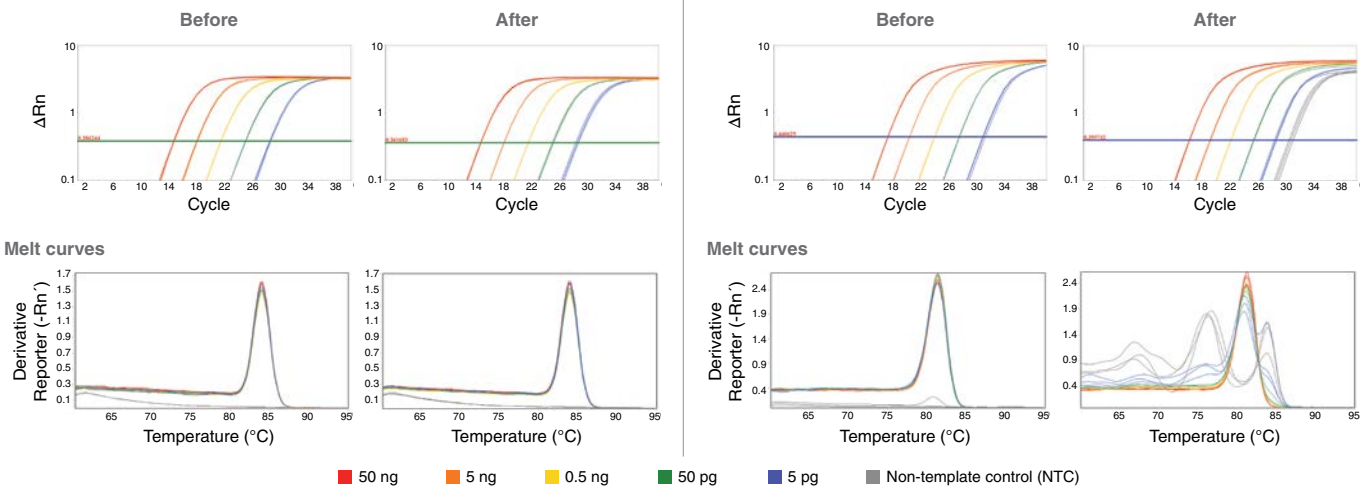
What is Luna WarmStart Reverse Transcriptase?

"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 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)

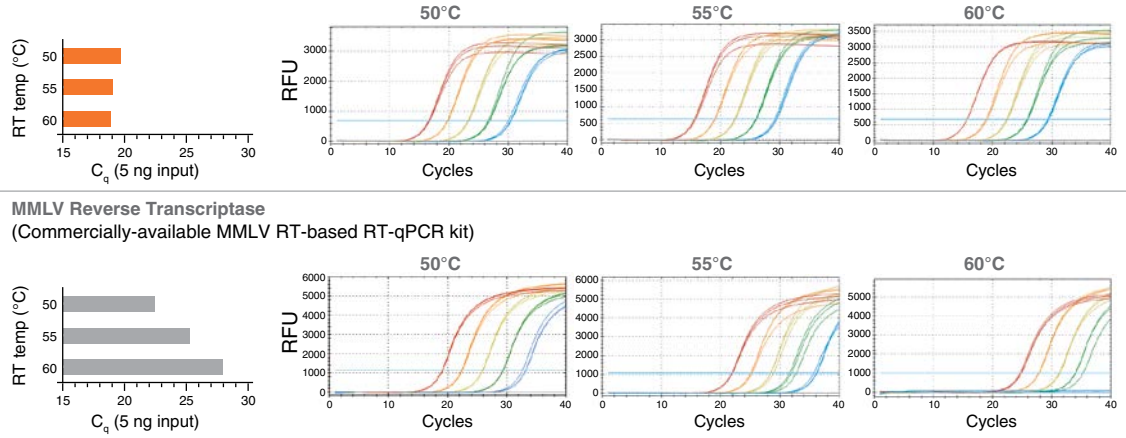


RT-qPCR targeting human ribosomal protein L32 was performed before and after a 24-hour incubation at room temperature, with triplicate reactions for a 5-log range of input human (Jurkat) total RNA and a non-template control. The Luna Universal One-Step RT-qPCR Kit featuring Luna WarmStart Reverse Transcriptase exhibited robust performance and no detectible 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.

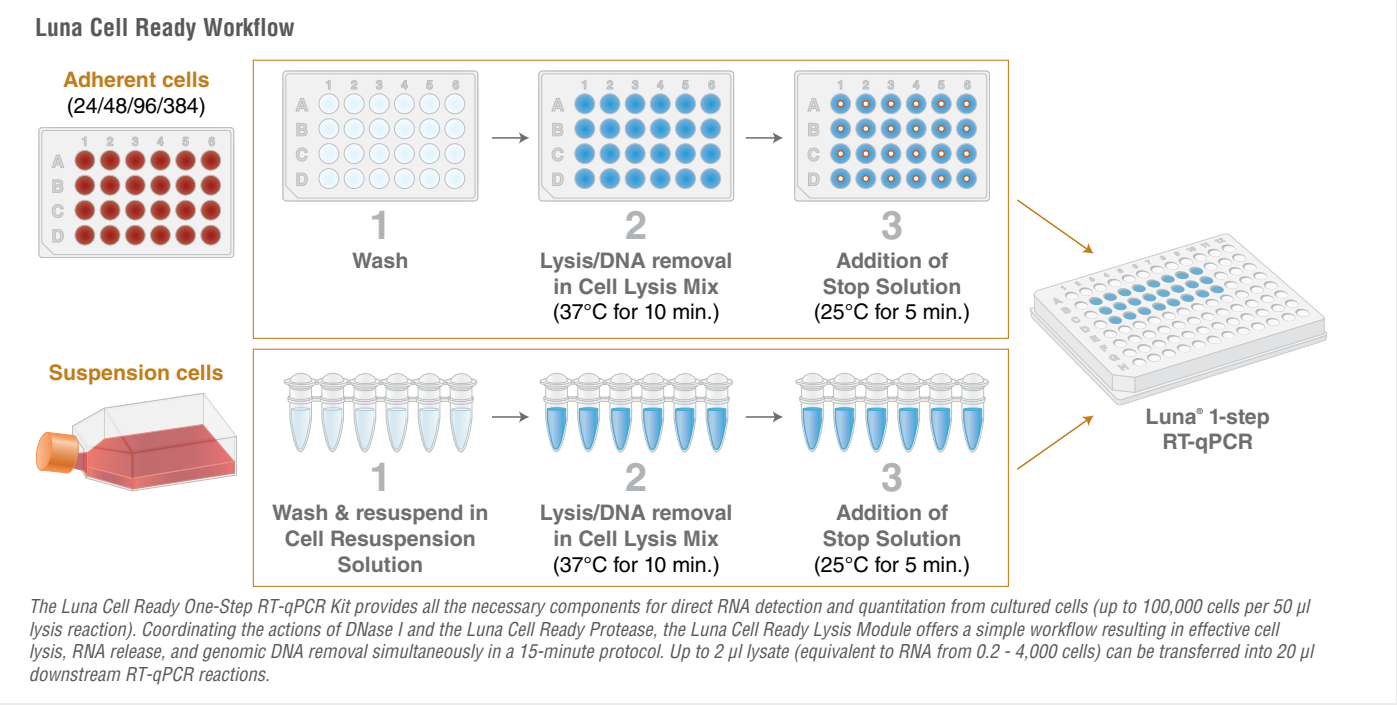
B. In contrast, a commercially available MMLV (recommended incubation temperature: 50°C) exhibited delayed (increased) C_q values, poorer performance, and loss of low-input detection at elevated temperatures, consistent with loss of RT activity.

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).

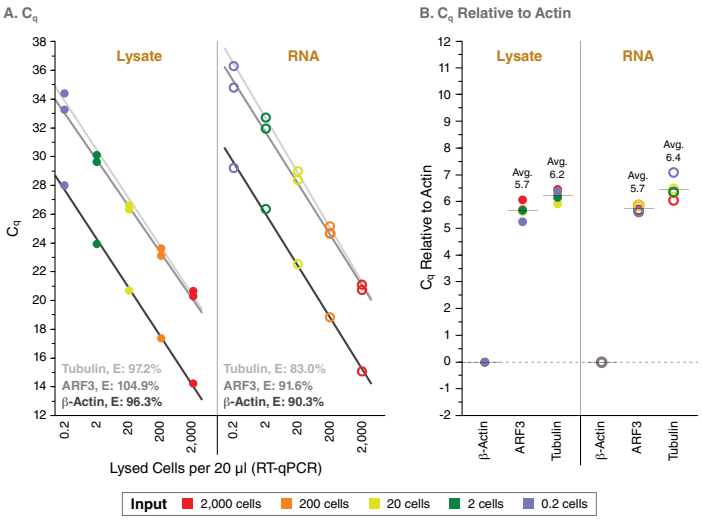
The Luna Cell Ready Lysis One-Step RT-qPCR Kit is available for both dye (NEB #3030) and probe (NEB #3031) detection methods. In addition, the lysis module can be purchased separately (NEB #3032).

- 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



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.

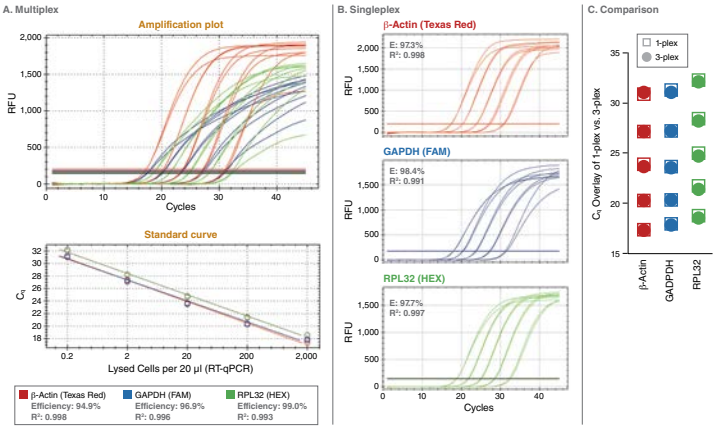


Serial dilutions of A549 cells (100,000–10) were lysed in 50 µl Luna Cell Ready lysis reactions (NEB #E3032) using standard reaction conditions (10 min lysis at 37°C, 5 min inactivation at 25°C). Alternatively, RNA was purified using a column-based RNA extraction kit.

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.

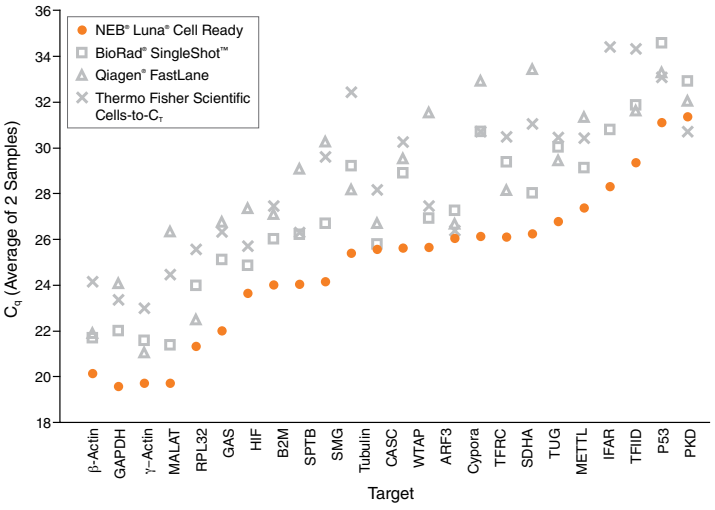
B. Cq relative to β-actin at each dilution (ΔCq (GOI)) was calculated from the data in (A) across 5-log cell inputs. The average Cqs are shown as bars.

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 ThermoFisher (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.

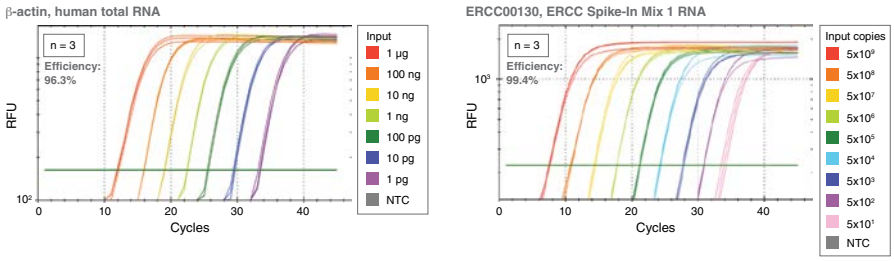
Speed up your Two-Step RT-qPCR: LunaScript RT SuperMix Kit.

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.

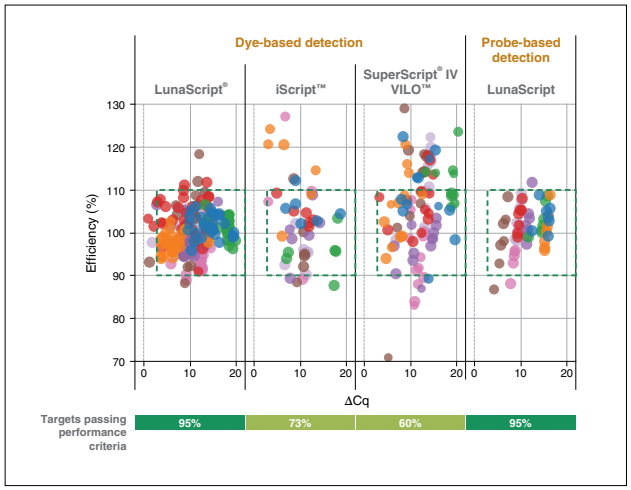
The cDNA products generated by LunaScript have been extensively evaluated in qPCR using the Luna qPCR Master Mixes (NEB #M3003/M3004). In combination, these products provide a two-step RT-qPCR workflow with excellent sensitivity and accurate, linear quantitations.

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



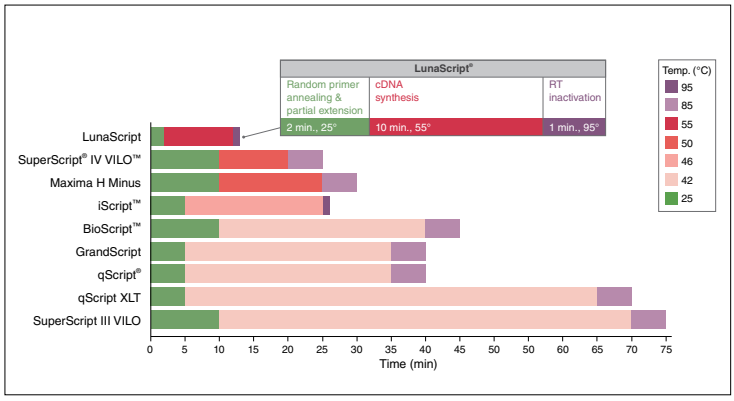
RNA was converted to cDNA using the 1X LunaScript RT SuperMix in 20 µl reactions using standard reaction conditions (25°C/2 min, 55°C/10 min, 95°C/1 min). cDNA was then quantitated by qPCR using the Luna Universal qPCR Master Mix (NEB #M3003) and 1 µl of cDNA product as template, with triplicate reactions 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. The LunaScript RT SuperMix Kit requires the shortest reaction time and tolerates elevated temperatures, reducing complications from RNA secondary structure.

Request a sample at
LUNAqPCR.com

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 (20 µl)
Luna Universal One-Step RT-qPCR Kit	E3005S/L/X/E	200/500/1,000/2,500 rxns (20 µl)
Luna Universal Probe One-Step RT-qPCR Kit	E3006S/L/X/E	200/500/1,000/2,500 rxns (20 µl)
LunaScript RT SuperMix Kit	E3010S/L	25/100 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)	M1800S/L	100/500 reactions (25 µl vol)
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**.

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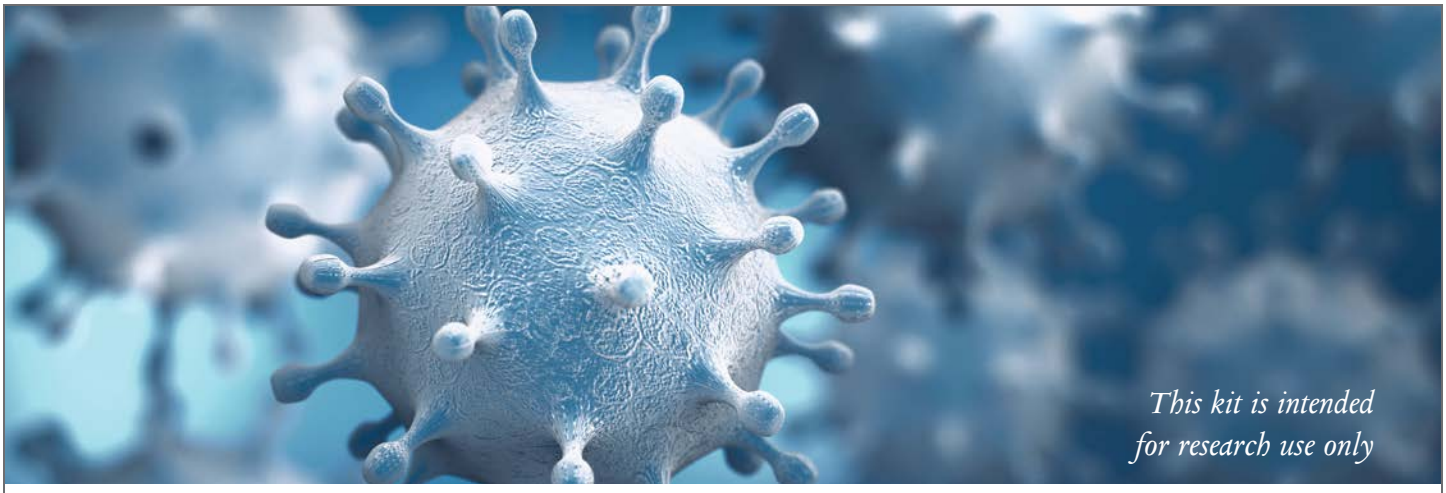


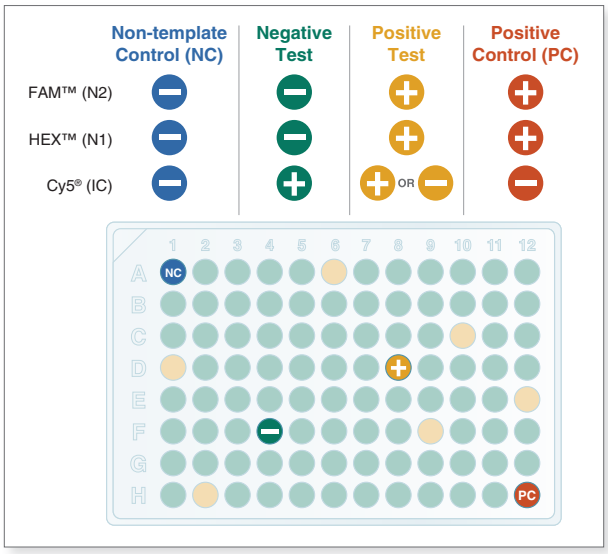
Luna® SARS-CoV-2 RT-qPCR Multiplex Assay Kit

The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit is a real-time RT-PCR assay for the qualitative detection of SARS-CoV-2 nucleic acid.

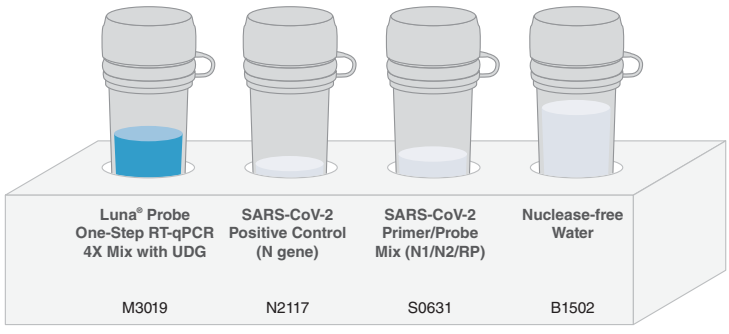
Highlights

- Multiplex detection of 2019-nCoV_N1 and 2019-nCoV_N2 targets and human RNase P gene enables high throughput workflows
- Reduce background amplification from genomic DNA using a modified RNase P Internal Control reverse primer to target an exon-exon boundary
- Increase assay sensitivity with 4X RT-qPCR mix allowing for more sample input
- Reduce risk of carryover contamination with thermolabile UDG and dUTP included in the master mix
- Supports sample pooling with minimal loss in assay sensitivity

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).



Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit components

For more details, visit **www.neb.com/E3019**

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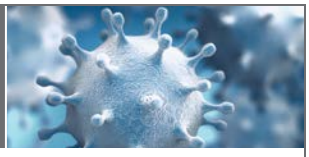
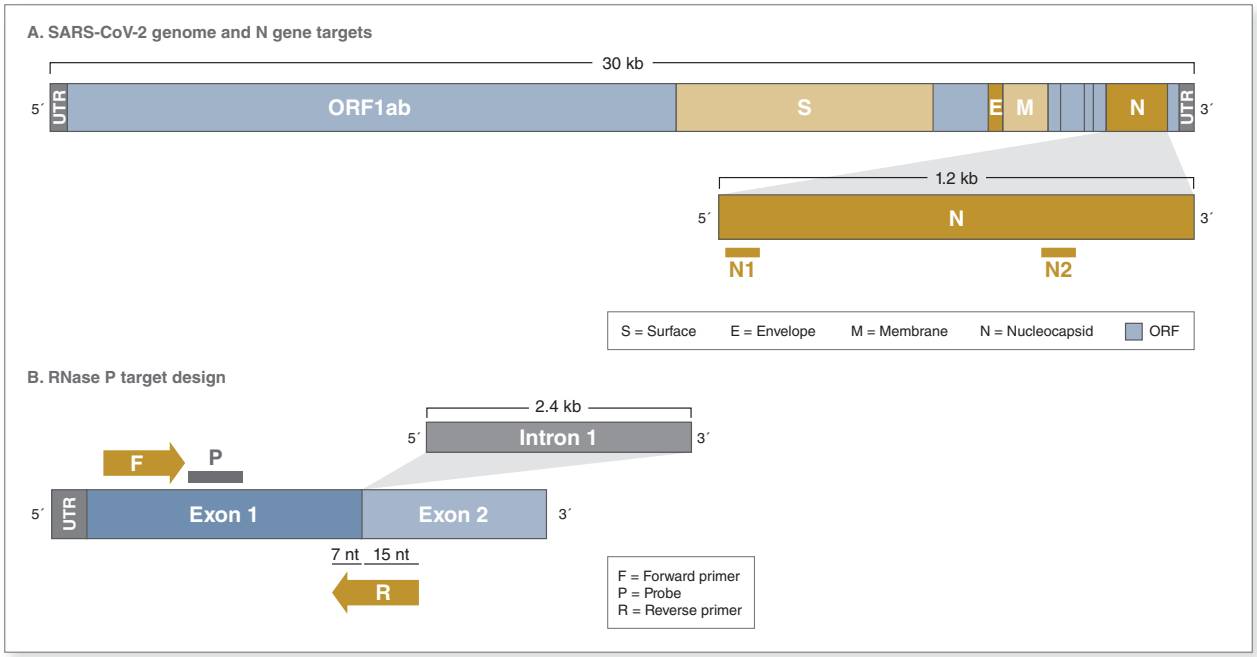
Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit



The SARS-CoV-2 Primer/Probe Mix in this kit contains primers and probes 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 simultaneous observation on two different channels of a real-time instrument. To ensure the integrity of the input material and absence of inhibition, an internal control (IC) primer and probe set, designed to amplify the human RNase P gene, is also included in the primer mix. The reverse primer of this target has been modified from the CDC design to spans an exon-exon junction to avoid amplification of human genomic DNA, which contains a 2.4 kb intron.

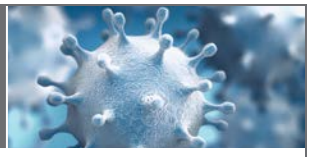
The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit detects two regions of the N gene and the human RNase P gene in a single reaction



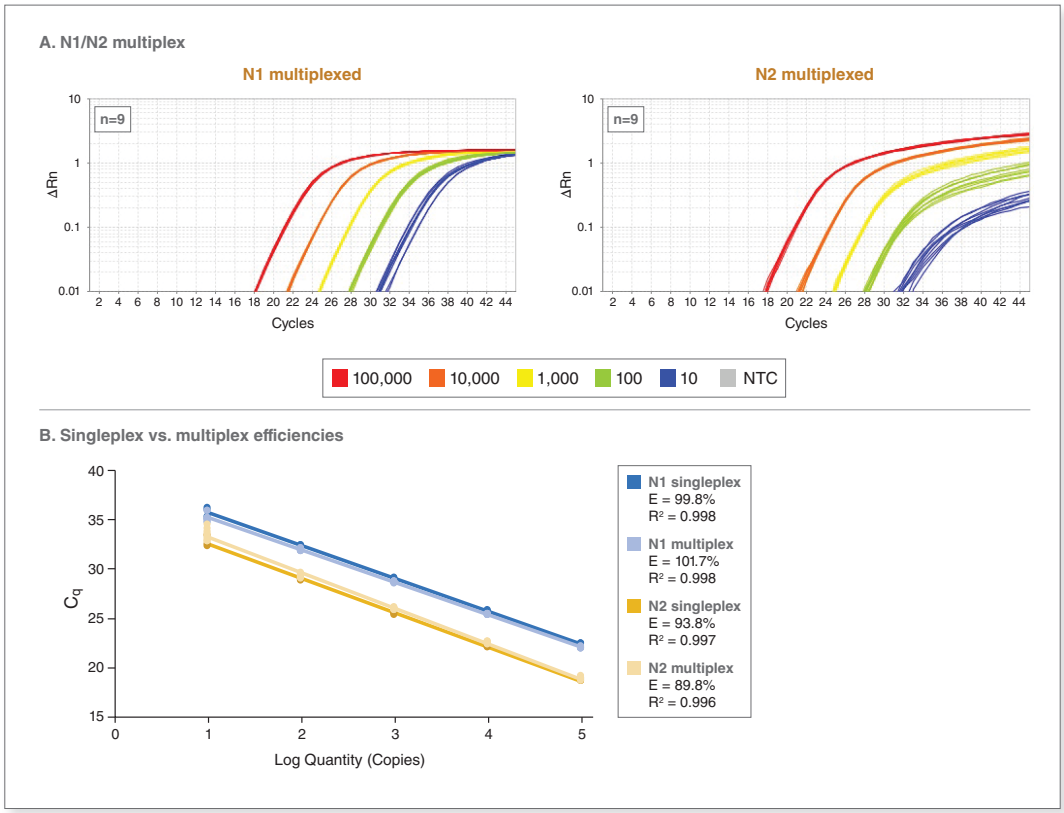
A. The two SARS-CoV-2 sequences are based on those provided by the CDC but modified to contain different fluorophores (N1: HEX, N2: FAM).
B. The RNase P internal control includes a Cy5 labeled probe and a re-designed reverse primer. This primer spans an exon-exon junction to avoid amplification of human genomic DNA which contains a 2.4 kb intron.

The Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) enables higher amounts of input material and supports sample pooling strategies, with minimal loss of sensitivity or specificity. It contains all necessary components for one-step RT-qPCR and is formulated with a unique passive reference dye that is compatible across a variety of instrument platforms, including those that require a high or low ROX reference signal. The reaction mix also features thermolabile UDG and dUTP for carryover prevention and a nonfluorescent visible tracking dye for monitoring reaction setup.

Learn more about how NEB is supporting COVID-19 research at **www.neb.com/COVID19**

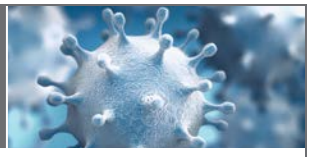


Multiplex detection of SARS-CoV-2 RNA 2019-CoV_N1 and 2019-nCoV_N2 targets

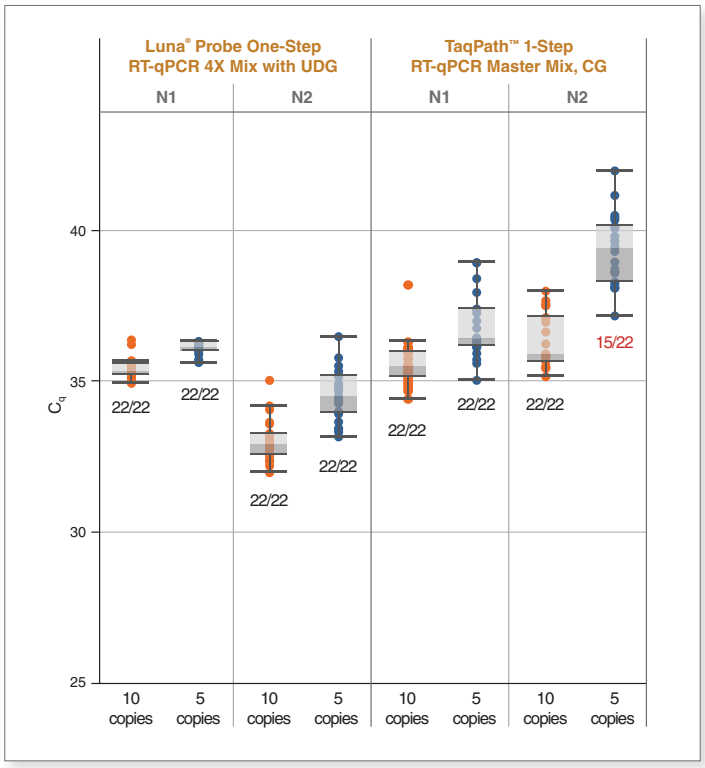


Amplification plots for multiplex targets 2019-nCoV_N1(HEX) and 2019-nCoV_N2 (FAM) (A) over a 5-log range of 100,000 – 10 copies of Twist Synthetic SARS-CoV-2 RNA Control 2 (Twist Biosciences, SKU 102024) diluted in 10 ng of Jurkat total RNA (BioChain, #R1255815-50) with 9 replicates at each concentration to examine reproducibility. Data was collected on an Applied Biosystems® 7500 Fast Real-Time instrument (96-well, 20 µl reactions). Singleplex vs multiplex detection of 2019-nCoV_N1 and 2019-nCoV_N2 (B) show similar C_q values for both single and multiplex workflows at each concentration and with similar overall amplification efficiency.

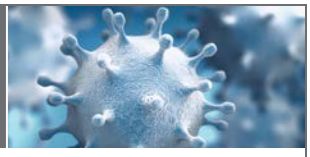
Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit



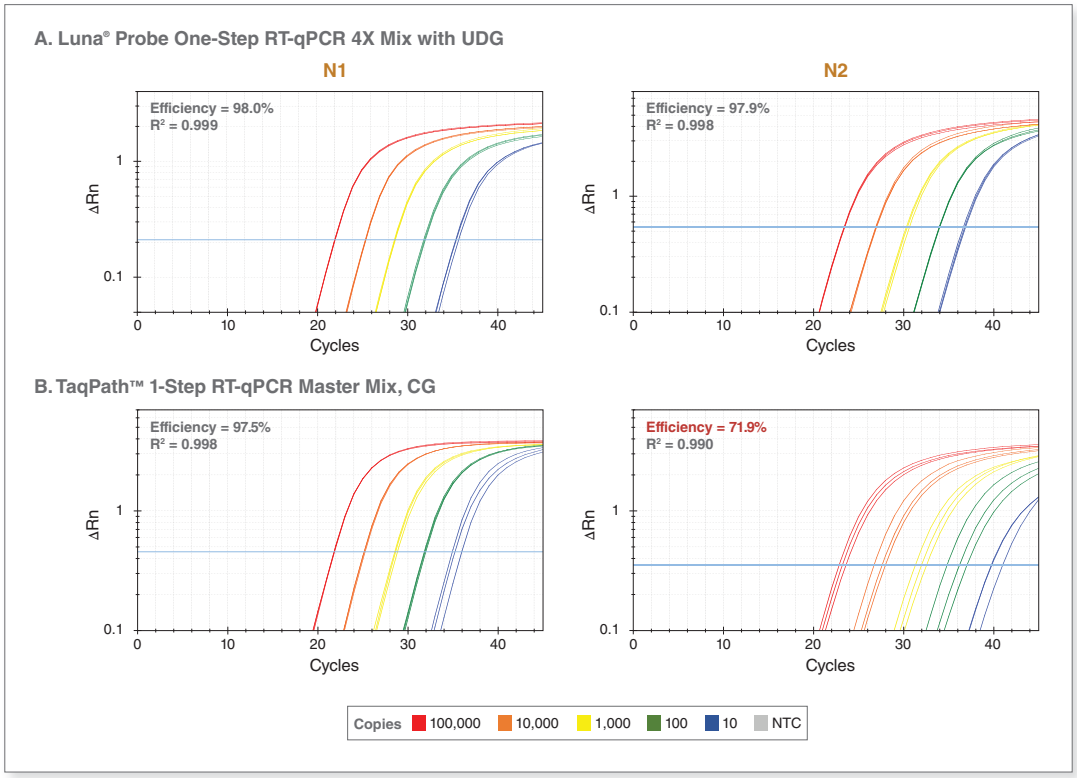
The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit demonstrates a lower limit of detection than TaqPath™ 1-Step RT-qPCR Master Mix, CG



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.



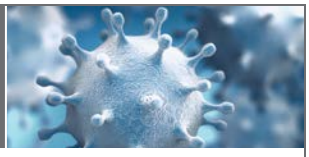
Improved performance on the SARS-CoV-2 N2 target from the Luna Probe One-Step RT-qPCR 4X Mix with UDG compared to the TaqPath 1-Step RT-qPCR Master Mix, CG



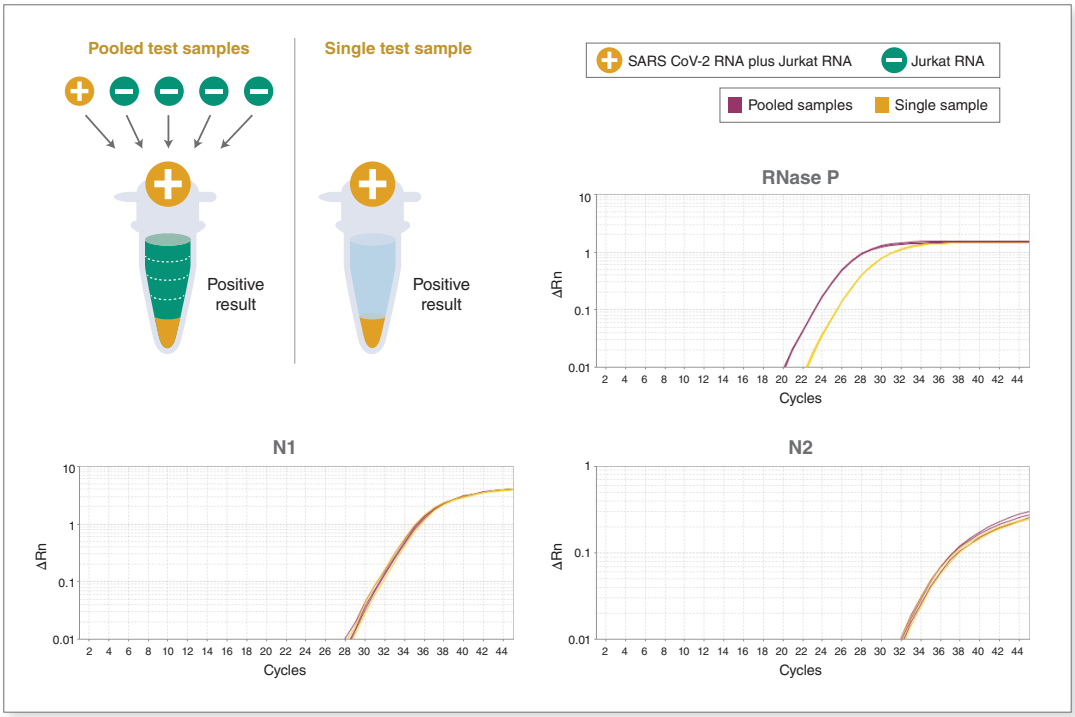
A. Singleplex RT-qPCR targeting 2019-nCoV_1 (HEX) and 2019-nCoV_N2 (FAM) was performed using the Luna Probe One-Step RT-qPCR 4X Mix with UDG, according to reaction and cycling conditions provided in the E3019 product manual.

B. Singleplex RT-qPCR targeting 2019-nCoV_N1 (FAM) and 2019-nCoV_N2 (FAM) was performed using TaqPath 1-Step RT-qPCR Master Mix, CG, as outlined in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel guidelines (https://www.fda.gov/media/134922/download). Performance was evaluated over a 5-log range of Twist Synthetic 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, both Luna and TaqPath mixes perform well with the N1 target. For the N2 target, even though 10 copies is in a detectable range for the TaqPath mix, substandard linearity is consistently observed, while the Luna mix exhibits strong linearity and faster C_q's.

Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit



Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit enables sample pooling of purified RNA



A pool containing five samples was prepared by combining 2 µl of one mock positive (10 copies of the Twist Synthetic SARS-CoV-2 RNA Control diluted in 10 ng of Jurkat total RNA) and 2 µl each of 4 mock negative samples (10 ng Jurkat total RNA only). Multiplex performance for the pooled sample (10 µl) was compared to an individual sample (2 µl, a total of 10 copies of N gene and 10 ng of Jurkat total RNA). Data was collected on an Applied Biosystems 7500 Fast Real-Time instrument (96-well, 20 µl reactions). The amplification curves for the N1 and N2 targets indicate similar C_q values from a positive sample whether assayed individually or as part of a pool. As expected, the RNase P signal from the pooled sample has an earlier C_q compared to the single sample since it contains 5 times the amount of human total RNA.

Ordering Information

PRODUCT	NEB #	SIZE	PRICE
Luna SARS-CoV-2 Multiplex Assay Kit	E3019S/L	96/480 reactions	295 € / 1.288 €
COMPONENTS SOLD SEPARATELY			
SARS-CoV-2 Positive Control (N gene)	N2117S	50 µl	70 €
Luna Probe One-Step RT-qPCR 4X Mix with UDG	M3019S/L/X/E	200/500/1,000/2,000 reactions	310 € / 698 € / 1.241 € / 2.190 €

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