Protocol for LyoPrime Luna® One-Step RT-qPCR Mix with UDG, 96-well plate (NEB #L4001P)

Overview

Please read all instructions before opening the moisture barrier foil pouch for this product.

Introduction

One-Step RT-qPCR is a convenient and powerful method for RNA detection and quantitation. The LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001P) is provided lyophilized in a 'ready-to-use' 96-well plate format and is shipped and stored at room temperature prior to use.

All necessary components required for a 20 µL one-step RT-qPCR assay are provided in each well of the 96-well plate, except primers, probe(s) and RNA template. The RT-qPCR mix is formulated with a unique passive reference dye compatible across various instrument platforms, including those requiring a high or low ROX reference signal. It also features dUTP and thermolabile UDG for carryover prevention and a non-fluorescent visible dye for monitoring experiment setup and rehydration. This visible dye does not overlap spectrally with fluorophores commonly used in qPCR and does not interfere with real-time detection.

This product is available as a single 96-well plate. It also can be customized to meet your specific application needs by contacting us.

Plate Information and Instrument Compatibility

LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG is supplied in a low profile 96-well x 0.1 ml semi-skirted PCR plate capped with robust optical lids in a tear-off format. The strip tubes are in a single-use yellow shell frame semi-skirted adaptor and sit in a cardboard insert. The 96-well plate is packaged in a foil pouch to protect the lyophilized reagent from atmospheric moisture. Please see the **Storage Section** for information on the stability of the plate both before and after the foil pouch has been opened. The cardboard insert can immediately be removed and recycled upon opening the pouch. The product is compatible across several common instrument platforms, including Bio-Rad® CFX96 Touch/Opus, Applied Biosystems® FAST Cyclers and Roche® Lightcycler®.

Plate usage: The twelve 8-well strip tubes are partially attached, allowing the use of the entire plate or partial consumption by separating one or more individual 8-well strip tubes after removal from the yellow shell frame adaptor. The 8-well strips and lids are labeled 1 to 12 at the bottom to provide a tracking system independent of the yellow shell frame adaptor. The yellow shell frame adaptor must be removed to separate one or more strip tubes, but the tubes can be clicked back into the adaptor if necessary. To release the tubes from the frame grid, set the plate on a flat surface and press down firmly on the four corners of the frame grid. Then, carefully lift the tubes from the grid. If a single strip tube or partial plate is used in combination with the yellow shell frame grid (optional), we recommend disposal of the yellow shell frame adaptor along with the PCR tubes post-amplification to avoid amplicon contamination events that could occur with additional tube/adaptor manipulation. Additional yellow shell frame adaptors can be purchased from BIOplastics (product code AB19805G).

Instrument compatibility: The yellow shell adaptor has a chamfered edge at the upper left corner (A1), which supports the use of the product on ABI/Life Technologies FAST Cyclers and robotic systems. The yellow shell frame provides rigidity to the plate, so it is highly recommended to use the adaptor for full plate runs in the ABI/Life Technologies FAST Cyclers. If the plate is used partially, individual 8-well strips can be used **with or without** the yellow shell frame. Note that removing tubes from the yellow shell frame adaptor may be recommended for thermocycling on some instrument platforms. Please refer to the table below for recommendations on using the yellow shell adaptor in common real-time PCR instruments.

Common Real-Time PCR Instruments	Yellow Shell Frame Adaptor Recommended	
	Full plate	**Partial plate**
Bio-Rad CFX96 Touch/Opus	No	No
Applied Biosystems 7500/7500 Fast	Yes	Optional
Applied Biosystems StepOne™/StepOnePlus™	Yes	Optional
Applied Biosystems QuantStudio® 3/5/6/7 Fast	Yes	Optional
Roche LightCycler 96	No	No

Storage

Before use, store the LyoPrime Luna Mix at room temperature (15°C to 25°C), unopened, in its original foil pouch. The product has a shelf life of 24 months when stored properly under these conditions. The foil pouch and desiccant inside protect the lyophilized reagent from atmospheric moisture. After opening the foil pouch, it is recommended to hydrate the lyophilized reagent intended for use within **1 hour**. Any unused strip tubes should immediately be placed back in the foil pouch with the desiccant, sealing the pouch securely and storing it at room temperature. Failure to do so will result in a gummy reagent that is difficult to rehydrate, and product performance may be impacted. It is recommended to use all strip tubes within **1 week** after opening the foil pouch for the best results.

Rehydration

Remove the optical lids and add liquid components indicated in the protocol section below. It is highly recommended to prepare an assay mix, including primers and probe(s), before dispensing into each well to eliminate volumetric errors and ensure proper rehydration of the Luna mix. The minimum initial resuspension volume for the 20 µL reaction is 5 µl. Rehydrate the lyophilized materials by adding the assay mix into each well and mix by pipetting up and down or by gentle vortex using a microplate shaker before adding the RNA template.

Reaction Setup

An example protocol for setting up a 20 µl reaction with the addition of 2 µl RNA template is given below.

1. Open the foil pouch by tearing along the pre-cut notch and remove the 96-well plate. Remove and recycle the cardboard insert if desired.

2. If using individual 8-well strip tubes OR for use on non-ABI instruments, remove the strip tubes from the yellow shell adaptor. To release the tubes from the frame grid, set the plate on a flat surface and press down firmly on the four corners of the frame grid. Then, carefully lift the tubes from the grid. Separate the desired number of 8-well strip tubes and place in a PCR rack. Immediately place any unused strip tubes back in the foil pouch with desiccant and seal securely. Click tubes back into the yellow shell adaptor if necessary. Please note that the yellow shell adaptor is not required for thermocycling on all instruments (see recommendations above).

3. Determine the total volume for the appropriate number of reactions, plus 10% overage, and prepare an assay mix of all components **except RNA template**, according to the table below.

COMPONENTS	20 µl REACTION	FINAL CONCENTRATION
LyoPrime Luna One-Step RT-qPCR Mix with UDG	-	1X
Forward Primer (10 µM)	0.8 µl	0.4 µM
Reverse Primer (10 µM)	0.8 µl	0.4 µM
Probe (10 µM)	0.4 µl	0.2 µM
Template RNA	variable	< 1 µg (total RNA)
Nuclease-free Water	to 20 µl	

4. Aliquot assay mix into the desired wells of the plate. For best results, ensure accurate and consistent pipetting volumes and minimize bubbles.

5. Mix the lyophilized pellets thoroughly but gently by pipetting up and down five times. Alternatively, vortex using a microplate shaker (30 seconds at ~1600 rpm). Collect liquid to the bottom of the tube by brief centrifugation if necessary.

6. Add RNA template to the plate. Cap the plate using the provided optical lids.

7. Spin tubes or plates briefly to remove bubbles and collect liquid (1 minute at 2,500–3,000 rpm).

8. Thermocycle according to the programming information below. After thermocycling and data generation are complete, discard completed reactions (tubes and yellow shell frame adaptor).

Programming the Real-Time Instrument

Use the All-Channel scan mode setting on the real-time instrument.

For faster results, the “Fast” ramp speed mode can be used where available (e.g., Applied Biosystems StepOnePlus, QuantStudio, 7500 Fast instruments).

CYCLE STEP	TEMPERATURE	TIME	CYCLES
Carryover Prevention	25°C	30 seconds	1
Reverse Transcription	55°C	10 minutes	1
Initial Denaturation	95°C	1 minute	1
Denaturation Extension	95°C 60°C	10 seconds 30* seconds (+ plate read)	45

* For Applied Biosystems real-time instruments (ABI), using a 60-second extension step can benefit some challenging targets.

Data Analysis

1. For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

2. After the run is complete, inspect the amplification plot to ensure that the baseline threshold was set within the PCR exponential phase and above any background signal.

Additional Considerations

Primer and probe design

We recommend using primer design software (e.g., Primer3) to select target, primer, and probe sequences to maximize amplification efficiency while minimizing nonspecific amplification and primer dimers. Luna qPCR is also compatible with commercially available qPCR assays. If designing primers manually, we encourage designing short amplicons (70 bp to 200 bp) with balanced GC content (40-60%). For primers and probes, aim for a T_m of approximately 60°C using Hot Start *Taq* settings in the NEB T_m calculator. Where possible, it is advisable to design primers across known splicing sites (exon-exon junctions) to prevent amplification from genomic DNA.