NEW ENGLAND Biolabs

Luna® Universal qPCR Master Mix Protocol (NEB #M3003)

Overview

This dye-based quantitative PCR (qPCR) protocol uses a convenient Luna® Universal master mix format that contains all qPCR components required for amplification and quantification of DNA, except primers and DNA template. The passive reference dye in the mix is compatible with a variety of real-time instruments, including those that require No ROX, Low ROX, or High ROX passive reference dye concentrations. This master mix also includes dUTP for carryover prevention and a non-fluorescent, inert blue tracking dye to monitor reaction setup. This dye does not spectrally overlap with fluorescent dyes used for qPCR and will not interfere with real-time detection.

Before starting

- Prepare DNA or cDNA of interest using desired DNA extraction and purification method.
- Make dilutions of DNA or cDNA to be used for the standard curve. These should be prepared fresh before each experiment and can be diluted in either water or TE.

Reaction Setup: For best results, we recommend running each DNA standard and sample in triplicate.

COMPONENT	20 µl REACTION	FINAL CONCENTRATION
Luna Universal qPCR Master Mix	10 µl	1X
Forward primer (10 µM)	0.5 µl	0.25 µM
Reverse primer (10 µM)	0.5 µl	0.25 µM
Template DNA	variable	< 100 ng
Nuclease-free Water	to 20 µl	

1. Thaw Luna Universal qPCR Master Mix and other reaction components at room temperature, then place on ice. After thawing completely, briefly mix each component by inversion, pipetting or gentle vortexing.
2. Determine the total volume for the appropriate number of reactions, plus 10% overage and prepare assay mix of all components except DNA template accordingly. Mix thoroughly but gently by pipetting or vortexing. Collect liquid to the bottom of the tube by brief centrifugation.
3. Aliquot assay mix into qPCR tubes or plate. For best results, ensure accurate and consistent pipetting volumes and minimize bubbles.
4. Add DNA templates to qPCR tubes or plate. Seal tubes with flat, optically transparent caps; seal plates with optically transparent film. Care should be taken to properly seal plate edges and corners to prevent artifacts caused by evaporation.
5. Spin tubes or plates briefly to remove bubbles and collect liquid (1 minute at 2,500–3,000 rpm).
6. Program real-time instrument with indicated thermocycling protocol (see table below). Ensure a plate read is included at the end of the extension step.

Use the SYBR® or SYBR/FAM scan mode setting on the real-time instrument.

We recommend using the “Fast” ramp speed where applicable (e.g., Applied Biosystems StepOnePlus®, QuantStudio®, 7500 Fast instruments).

CYCLE STEP	TEMPERATURE	TIME	CYCLES
Initial Denaturation	95°C	60 seconds	1
Denaturation	95°C	15 seconds	40-45
Extension	60°C	30 seconds (+plate read)	
Melt Curve	60-95°C*	various	1

*Follow real-time instrument recommendations for melt curve step.