qLUMEN™ RT-qPCR Kit with probe

RPCR-KGH-100 / RPCR-KGL-100

Introduction

The qLUMEN™ RT-qPCR Kit allow efficient cDNA synthesis and qPCR in a single tube. The kit includes a qPCR master mix supplied in a 2X concentration to perform real-time PCR. The qPCR master mix contains all the reagent (except PCR primers and template) needed for running PCR reactions. In addition, a separate RT mix that comprises a balanced mixture of both RTase and RNase Inhibitor is also provided.

ROX™ Reference Dye is provided in a separate tube. For certain real-time cyclers, the presence of ROX™ passive reference dye in real-time PCR compensates for non-PCR-related variations in fluorescence detection.

RT-PCR is used to amplify double-stranded DNA from single-stranded RNA templates. In the RT step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template. In the first cycle of the PCR step synthesis, Taq DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. During subsequent rounds of cycling the DNA polymerase exponentially amplifies the double-stranded DNA template.

Features

- ✓ Higher specificity, sensitivity, and yield
- ✓ Compatible with most real-time PCR instruments

Applications

- ✓ Detection and quantification of DNA and cDNA targets
- ✓ Gene expression
- ✓ For use with standard and fast qPCR platforms
- ✓ High throughput applications

Quality Control

Functionally tested in RT qPCR on Applied Biosystems StepOne® Real-Time PCR System. Tested for activity, processivity, efficiency, sensitivity and heat activation.

Storage

The Kit should be stored at -20°C upon receipt. Avoid repeated freezing and thawing.

Kit contents

Item	Volume
qLUMEN™ RT-qPCR (2X)	1 ml
RT mix	100 µl
ROX™ reference Dye	30 µl
RNase-free Water	1 ml

Product use limitation

This product is developed, designed and sold exclusively for research purposes and use only. The product is not intended for diagnostics or drug development, nor is it suitable for administration to humans or animals.

Protocol

Before starting

- ✓ Add 30 µl ROX™ Reference Dye to 1 ml qLUMEN™ RT-qPCR (2X).
- ✓ Mix thoroughly, store at −20°C and protect from light.

1. Thaw kit components, template DNA, primers and nuclease-free H_2O on ice. Mix each solution well.

The following protocol is recommended for a 20 µl reaction volume

2. Set up the following reaction mixture

Component	Reaction volume 20 µL	Final concentration
Forward Primer	X µL	100-400 nM(1)
Reverse Primer	X µL	100-400 nM(1)
RNA template	X µL	0.01 pg to 1 µg (2)
qLUMEN™ RT-qPCR (2X)	10 µL	1X
RT mix	1µl	1X
Nuclease-Free Water to final volume of	20 µL	-

(1) Too high primer concentrations result in unspecific amplification and should be avoided.
(2) For optimal performance, use 1 pg – 1 µg Total RNA, or >0.01 pg mRNA.

3. Mix reagents completely, and then transfer to a thermocycler.
4. Program the appropriate PCR cycling protocol on your real-time PCR instrument

Suggested thermal cycling conditions

Step	Temperature	Time	Cycles
Reverse Transcription	45-55°C	10 min	1
Initial activation	95°C	2 min	1
Denaturation	95°C	5 s	40
Annealing/Extension*	60-65°C	20-30 s	

* Do not exceed 30 seconds. Do not use temperatures below 60°C.

- ✓ *As with all Real-Time PCR reactions, conditions may need to be optimized. You may be able to adjust your PCR conditions to optimize reaction*