qLUMEN™ RT-qPCR Kit with probe

RPCR-KPH-100 / RPCR-KPL-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. The ROX™ dye provides an internal reference to which the reporter-dye signal can be normalized during data analysis.
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 doublestranded DNA template.

Features

- ✓ Higher specificity, sensitivity, and yield
- ✓ Compatible with most real-time PCR instruments
- ✓ For use on a wide range of probe technologies including Taqman®, Molecular Beacons® and Scorpion® probes

Applications

- ✓ qLUMEN™ RT-qPCR based on specific probes
- ✓ Detection and quantification of DNA and cDNA targets
- ✓ Gene expression
- ✓ For use with standard and fast qPCR platforms
- ✓ High throughput applications

Quality Control

Mix Functionally tested in RT qPCR based on specific probe. 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
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

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)]
Specific Probe	X µL	200 nmM
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)] 1 µl is recommended; 2 µl may increase primer dimers, but improves Ct

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	50°C	10 min	1
Initial activation	95°C	3 min	1
Denaturation	95°C	10 s	40-45
Annealing/Extension*	60-68°C	30 s	

* Do not use annealing temperatures below 60°C. Recommendation is primer Tm +2°C or use gradient PCR to optimize the annealing temperature.

- ✓ 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
- ✓ For efficient amplification under fast cycling conditions use amplicon lengths between 80 bp and 200 bp
- ✓ The shorter the amplicon length the faster the reaction can be cycled. Use maximum 400 bp amplicons
- ✓ Primers should have a predicted melting temperature of around 60°C
- ✓ For TaqMan® probes choose probe close to 5’ primer, avoid terminal guanosine residues