

RealScript™ One-Step qRT-PCR Kit (For Probe System w/ROX)

Description

RealScript™ One-Step qRT-PCR Kit (For Probe System w/ROX) is designed for the reverse transcription and real-time PCR amplification of a specific target RNA from either total RNA or mRNA. RealScript™ One-Step qRT-PCR Kit (For Probe System w/ROX) combines the first-strand cDNA synthesis (reverse transcription) reaction and real-time PCR reaction (For Probe System w/ROX) in the same tube, simplifying reaction setup and reducing the possibility of contamination. This one-tube system provides sensitive, quick, precise and reproducible analysis of gene expression.

This kit consists of two major components: RealScript™ Reverse Transcriptase and RealSens™ Real-Time PCR Master Mix (For Probe System w/ROX). RealScript™ Reverse Transcriptase is a unique enzyme, different from the reverse transcriptases of Moloney Murine Leukemia Virus (MMLV) or Avian Myeloblastosis Virus (AMV). As a version of mutated MMLV, RealScript™ Reverse Transcriptase is genetically engineered to increase half-life, reduce RNase H activity, increase thermal stability, increase specificity of RT, provide more full-length product and lead to the highest cDNA yield of all RTs. RealScript™ Reverse Transcriptase is ideal for cDNA synthesis using a gene-specific primer, random primer, or either total RNA or poly(A)+-selected RNA primed with oligo(dT). RealSens™ Real-Time PCR Master Mix (For Probe System w/ROX) is highly sensitive and optimized for use with any real-time PCR cyclers using sequence-specific probes format. Since RBC SuperTaq® HotStart DNA Polymerase activates only after heating, it prevents the formation of mis-primed products and primer-dimers at low temperature during qPCR setup and the initial qPCR cycle.

This optimized RealScript™ One-Step qRT-PCR Kit (For Probe System w/ROX) contains all the factors needed for reverse transcription and real-time PCR amplification. Real-time RT-PCR can be done easily by simply adding template RNA, probe and primers into the tube. This one-step system not only eliminates any nonspecific amplification products and reduces background smear, but also ensures highly sensitive and reproducible qRT-PCR. Since it's a one-tube system, both cDNA synthesis and qPCR amplification can be processed in a single tube. The simple procedure makes high-throughput analysis possible.

Features

- Optimized, ready-to-use mixture format makes cDNA synthesis and qPCR amplification simple and easy.
- One-tube system provides sensitive, quick, precise and reproducible analysis of gene expression.
- Both cDNA synthesis and real-time PCR amplification can be processed in a single tube.
- Highly-sensitive detection of even low copy numbers of target genes.

Specifications

Cat. No.	Product Name	Specification
RR161	RealScript™ One-Step qRT-PCR Kit, (For Probe System w/ROX) 50 reactions	2X RealScript™ One-Step qRT-PCR Master Mix (For Probe System w/ROX): 625µl Sterilized ddH ₂ O: 1ml
RR162	RealScript™ One-Step qRT-PCR Kit, (For Probe System w/ROX) 100 reactions	2X RealScript™ One-Step qRT-PCR Master Mix (For Probe System w/ROX):1.25ml Sterilized ddH ₂ O: 2ml
RR161S	RealScript™ One-Step qRT-PCR Kit, (For Probe System w/ROX) 10 reactions	2X RealScript™ One-Step qRT-PCR Master Mix (For Probe System w/ROX): 125µl Sterilized ddH ₂ O: 200ul

Content

2X RealScript™ One-Step qRT-PCR Master Mix (For Probe System w/ROX) Contains:

- RealScript™ Reverse Transcriptase
- RBC SuperTaq® HotStart DNA Polymerase
- RBC Taqman real-time PCR buffer
- ROX passive reference dye
- dNTP mix including dATP、dCTP、dGTP、dTTP
- 5mM MgCl₂

Quality Control

Specificity and reproducibility of RealScript™ One-Step qRT-PCR Kit (For Probe System w/ROX) is tested in reproducibility assay: parallel 25µl reactions containing 2ul of human total RNA from embryonic kidney cell lysate and 0.6 µM primers, specific for d(T)18. After 35 cycles, B2M transcript is detected. The length of cDNA achieved is verified as 248 bp by electrophoresis and DNA sequencing.

Applications

- Gene expression analysis of RNA targets
- Detection of gene regulation

Shipping and Storage Conditions

RealScript™ One-Step qRT-PCR Kit (For Probe System w/ROX) is shipped on dry ice and should be stored immediately upon receipt at -20°C in a constant temperature freezer. With proper storage, this kit can be stored for up to 12 months without showing any deduction in performance and quality.

Protocol

General Reaction Conditions

Our protocol is for a reaction size of 25ul. This protocol serves only as a guideline for real-time RT-PCR amplification. Optional reaction conditions may vary and must be individual determined.

1. Add the following components to a sterile microtube on ice:

Component	Volume/ Reaction	Final Concentration
2X RealScript™ One-Step qRT-PCR Master Mix	12.5 µl	1X
Forward Primer (5-10µM)	Variable	0.6-1.0µM
Reversed Primer (5-10µM)	Variable	0.6-1.0µM
Probe (5-10µM)	Variable	0.2-0.4µM
Template RNA (100-200ng)	2-5ul	
Sterilized ddH ₂ O	Add to 25.0µl	

2. Mix above components thoroughly by pipetting up and down and dispense the 25µl of mixture into PCR tubes or plates.

3. **PCR tubes are kept on ice until the real-time thermal cycle has reached 48 °C.**

4. Suggested reaction parameters are as below.

Segment	Number of Cycles	Temperature	Time
1*	1	48°C	30 minutes
2	1	95°C	10 minutes
3	40	94°C	20-30 seconds
		55~65°C	>30 seconds
Optional**	40	72°C	1 minute

* Segment 1 is for Reverse Transcription.

**Suggested reaction parameters are designed for PCR products less than 300bp. For PCR products larger than 300bp, optional temperature 72°C is recommended. It takes around 1 minute at 72°C for 1kb.

5. Place the PCR tubes or PCR plates in the thermal cycle and start the real-time RT-PCR program.

Notes

1. Use disposable tips containing hydrophobic filters to minimize cross-contamination.
2. This product is developed, designed and sold for research use only. Not for use in diagnostic or therapeutic procedures.