



RealScript™ One-Step RT-PCR Kit

Description

RealScript™ One-Step RT-PCR Kit is designed for the reverse transcription and PCR amplification of a specific target RNA from either total RNA or mRNA. RealScript™ One-Step RT-PCR Kit combines the first-strand cDNA synthesis (reverse transcription) reaction and PCR reaction in the same tube, simplifying reaction setup and reducing the possibility of contamination. This one-tube system provides sensitive, quick and reproducible analysis of even rare RNA.

This kit consists of two major components: RealScript™ Reverse Transcriptase and RealSens™ HotStart DNA Polymerase. 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™ HotStart DNA Polymerase is ideal for DNA fragment amplification. Since RealSens™ HotStart DNA Polymerase activates only after heating, it prevents the formation of mis-primed products and primer-dimers at low temperature during PCR setup and the initial PCR cycle.

This optimized RealScript™ One-Step RT-PCR Kit contains all the factors needed for reverse transcription and PCR amplification. RT-PCR can be done easily by simply adding template RNA and primers to the tube. This one-step system not only eliminates any nonspecific amplification products and reduces background smear, but also ensures highly sensitive and reproducible RT-PCR. Since it is a one-tube system, both cDNA synthesis and PCR amplification can be processed in a single tube. The simple procedure makes high-throughput analysis possible.

Cat. No.	Product Name	Specification
RR101	RealScript™ One-Step RT-PCR Kit, 50 reactions	2X RealScript™ One-Step RT-PCR Master Mix: 625µl Sterilized ddH ₂ O: 1ml
RR102	RealScript™ One-Step RT-PCR Kit, 100 reactions	2X RealScript™ One-Step RT-PCR Master Mix: 1.25ml Sterilized ddH ₂ O: 2ml
RR101S	RealScript™ One-Step RT-PCR Kit, 10 reactions	2X RealScript™ One-Step RT-PCR Master Mix: 125µl Sterilized ddH ₂ O: 200ul



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Features

- Optimized, ready-to-use mixture format makes cDNA synthesis and PCR amplification simple and easy.
- One-tube system provides sensitive, quick and reproducible analysis of even rare RNA.
- Both cDNA synthesis and PCR amplification can be processed in a single tube.
- Ideal for analysis of large numbers of samples.

Content

2X RealScript™ One-Step RT-PCR Master Mix Contains:

- RealScript™ Reverse Transcriptase
- RealSens™ HotStart DNA Polymerase
- dNTP mix including dATP、dCTP、dGTP、dTTP
- 5mM MgCl₂

Quality Control

Specificity and reproducibility of RealScript™ One-Step RT-PCR Kits are 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

- Virus detection
- Single-cell RT-PCR
- Gene-expression analysis

Shipping and Storage Conditions

RealScript™ One-Step RT-PCR Kit is shipped on dry ice and should be stored immediately upon receipt at -20°C in a constant temperature freezer. With proper storage, RealScript™ One-Step RT-PCR 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 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 RT-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
Sterilized ddH ₂ O	Add to 23.0µl	

2. Mix above components thoroughly by pipetting up and down and dispense the 23µl of mixture into PCR tubes or plates.
3. Add 2µl of the RNA and mix carefully by pipetting up and down.
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	35~40	94°C	30 seconds
		50~68°C	30 seconds
		72 °C *	1 minute

* It takes around 1 minute at 72°C for 1kb.

5. **PCR tubes are kept on ice until the thermal cycle has reached 48 °C.**
6. Place the PCR tubes or PCR plates in the thermal cycle and start the 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.