One Step RT-PCR Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0400101	50 rxn of 50 µl	1.25 ml One Step Mix 125 µl RT-RI Blend
BR0400102	100 rxn of 50 µl	2 × 1.25 ml One Step Mix 2 × 125 µl RT-RI Blend
BR0400103	500 rxn of 50 µl	10 × 1.25 ml One Step Mix 10 × 125 µl RT-RI Blend

COMPONENT	COMPOSITION
One Step Mix	Proprietary 2× buffer composition including Hot Start *Taq* DNA Polymerase, dNTPs, enhancers and stabilizers
RT-RI Blend	Proprietary 20× blend of efficient thermostable Reverse Transcriptase and unique Ribonuclease Inhibitor
STORAGE	-20°C (until expiry date – see product label)

FEATURES

- Efficient thermostable Reverse Transcriptase and RNase Inhibitor providing high cDNA yields
- Unique Hot Start *Taq* DNA Polymerase in a mix with high-quality dNTPs
- PCR enhancers allowing sensitive low background amplification

APPLICATIONS

- One-step RT-PCR
- Virus detection
- Amplification of GC-rich and complex templates

DESCRIPTION

biotechrabbit™ One Step RT-PCR Kit provides an easy and efficient way to perform both reverse transcription of RNA and PCR amplification of cDNA in one step. Only RNA template, primers and PCR-grade water are added. The 20× RT-RI Blend, which contains a blend of an efficient thermostable reverse transcriptase and a proprietary Ribonuclease Inhibitor, ensures high yields of cDNA.
The 2× One Step Mix contains unique Hot Start *Taq* DNA Polymerase, dNTPs, $MgCl_2$ and stabilizers in an optimized buffer and provides high PCR product yields with minimal background even when using low-abundance and difficult templates. PCR enhancers included in the mix allow efficient amplification of complex templates including GC or AT-rich sequences.

PROTOCOL

Prevention of reaction contamination

When assembling the amplification reactions, care should be taken to eliminate the possibility of contamination with undesired DNA.
RNase contamination is an exceptional concern when working with RNA. RNase A, providing most threat to RNA integrity, is a highly stable contaminant of any laboratory. To prevent RNA from degradation and to minimize possibility of contamination One Step RT-PCR; follow the guidelines below:

- Use separate clean areas for preparation of the samples and the reaction mixture.
- DEPC-treat all tubes and pipette tips or use certified nuclease-free labware with aerosol filters.
- Wear fresh gloves when handling RNA and all reagents.
- Always assess the integrity of RNA prior to One Step RT-PCR in denaturing agarose gel electrophoresis.
- Use only water and reagents that are free of DNA, DNAses and RNAses.
- With every One Step RT-PCR setup, perform a contamination control reaction that does not include template DNA.

BASIC PROTOCOL

- The mixes are designed to be used without any optimization as they have all necessary reaction components in optimal amounts for successful One Step RT-PCR.
- Thaw on ice and mix all reagents well. Keep all reagents and reactions on ice.
- To use time and reagents effectively, always prepare master mix for multiple reactions by mixing water, RT-RI Blend and One Step Mix.
- Pipet the master mix into thin-walled 0.2 ml PCR tubes.
- Add template and primers separately if they are not used in all reactions.

COMPONENT	VOLUME	FINAL CONCENTRATION
One Step Mix, 2×	25 µl	1×
RT-RI Blend, 20×	2.5 µl	1×
Forward Primer (10 µM)	2 µl	0.4 µM
Reverse Primer (10 µM)	2 µl	0.4 µM
RNA Template	0.1–1 µg total RNA or 10–500 ng mRNA	
Too much template increases the background, too low template amounts reduce the PCR accuracy		
PCR Grade, RNAse-free Water	Variable	
Total volume	50 µl	

- Mix and centrifuge briefly to collect the liquid in the bottom of the tube.
- Place in the PCR cycler.

CYCLING PROGRAM

STEP	TEMPERATURE	TIME	CYCLES
cDNA synthesis	45-55°C	10–20 min	1
Initial activation	95°C	2 min	1
Denaturation	95°C	10 s	35–40
Annealing	55°C	10 s	35–40
	Approximately 5°C below T_m of primers		
Extension	72°C	30–60 s/kb	35–40
Final extension	72°C	5 min	1
	To extend all incomplete PCR products		
Storage in the cycler	4°C	Indefinitely	1

- Add loading dye solution (see DNA Loading Dye, 6×, cat. no. BR0800301) to the reactions to analyze PCR products on a gel or store them at −20°C.

CERTIFICATE OF ANALYSIS

Functional Assay:
One step RT-PCR using eukaryotic total RNA as a template.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

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Certified Quality-System mdc ISO 13485 ISO 9001

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