

1-Step RT-PCR Master Mix

LOT: See product label EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT		
BR0400102	100 rxn of 50 µ	I 2 × 1.25 ml 1-Step Master Mix		
		2 × 125 μl RT-RI Blend		
COMPONENT	CO	COMPOSITION		
1-Step Master Mix	Prop	Proprietary 2X buffer composition including Hot Start Taq DNA		
	Poly	merase, dNTPs, enhancers and stabilizers		
RT-RI Blend	Prop Tran	Proprietary 20X blend of efficient thermostable Reverse		
	Trui			
STORAGE	-30	-30°C to -10°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[™] 1-Step Master Mix 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 20X RT-RI Blend, which contains a blend of an efficient thermostable reverse transcriptase and a proprietary Ribonuclease Inhibitor, ensures high yields of cDNA.

The 2X 1-Step Master Mix contains unique Hot Start *Taq* DNA Polymerase, dNTPs, MgCl₂ 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 1-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			
1-Step Master Mix	25 µl	1×			
20X RT-RI Blend	2.5 μl	1×			
Forward Primer (10 µM)	2 µl	0.4 µM			
Reverse Primer (10 µM)	2 µl	0.4 µM			
DNA Tomplete	0.1–1 µg total RNA or				
RNA Template	10–500 ng mRNA				
Too much template increases the background, too low template amounts reduce the PCR accuracy					
PCR Grade, RNase-free	Variable				

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 6X DNA Loading Dye, cat. no. BR0800301) to the reactions to analyze PCR products on a gel or store them at -20°C.

1-Step RT-PCR Master Mix

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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