5X UniFi™ 1-Step qRT-PCR Probe Master Mix

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0504601	250 rxn of 20 µl	1 ml 5X UniFi™ 1-Step qRT-PCR Probe Master Mix
BR0504602	1000 rxn of 20 µl	4 × 1 ml 5X UniFi™ 1-Step qRT-PCR Probe Master Mix

COMPONENT	COMPOSITION
UniFi™ 1-Step qRT-PCR Probe Master Mix	5X concentrated formulation of a UniFi™ 1-Step qRT-PCR Probe Master Mix

STORAGE Store at −20 °C until expiry date.

FEATURES

- Best in-class performance for both single and multiplex detection
- Convenient all-in-one master mix
- High specificity and sensitivity across a wide range of sample sources

APPLICATIONS

- One step qRT-PCR from mRNA, total RNA and viral RNA targets
- For use with standard and fast qPCR platforms
- Single and multiplex qRT-PCR reactions

DESCRIPTION

biotechrabbit™ UniFi™ 1-Step qRT-PCR Probe Master Mix provides outstanding performance for real-time PCR quantification of RNA templates, including mRNA, total RNA and viral RNA from a wide range of targets. The master mix ensures high specificity and sensitivity in single and multiplex detection, making it the choice for extremely low-copy-number targets in pathogen detection.
The mix is provided as a 5X concentrated master mix offering flexibility in pipetting schemes for template input in various PCR applications. UniFi 1-Step qRT-PCR Probe Master Mix uses proprietary reverse transcriptase technology and buffer chemistry for efficient cDNA synthesis and qPCR in a single tube.

Info: Recommended annealing temperature is 2°C above primer Tm (use gradient PCR to optimize the annealing temperature).

PROTOCOL

Notes

- 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, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/).
- For TaqMan® probes choose probe close to 5' primer, avoid terminal guanosine residues.

Prevention of reaction contamination

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 RT-PCR in denaturing agarose gel electrophoresis.
- Use only water and reagents that are free of DNA, DNAases and RNases.
- With every One Step RT-PCR setup, perform a contamination control reaction without template DNA.

Basic Protocol

- Keep the master mix protected from light until you use it.
- Aliquot the master mix to minimize freeze-thaw cycles and light exposure.
- Thaw on ice and mix very well all reagents. Assemble and keep all reactions on ice.
- Use only high quality optically clear reaction plates and seals designed for fluorescence applications.
- Do not use corner wells or use a more robust seal.
- Reserve plate positions for positive (control DNA) and negative (water or buffer) controls.
- First pipette the primer mixture, then add the template and last the Master Mix.
- Before preparing mixes, calculate the volume needed according to the reaction number plus one extra.
- To have a better correlation, run the reactions in triplets.

COMPONENT	VOLUME	FINAL CONCENTRATION
Primer Mix (Reverse and Forward)	Variable	100- 400 nM
Too high primer concentrations result in unspecific amplification and should be avoided.		
Specific Probe	Variable	200 nM
Template RNA	Variable	0.01 pg to 1 µg
Use 1 pg – 1 µg Total RNA, or >0.01 pg mRNA		
5X UniFi™ 1-Step qRT-PCR Probe Master Mix	4 µl	1×
Nuclease free water	Variable	
Total volume	20 µl	

- Gently mix the reactions without creating bubbles (do not vortex). Bubbles will interfere with fluorescence detection. Place the reaction into the PCR cycler.

CYCLING PROGRAM

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	

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

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Mix tested functionally in qRT-PCR.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)
Sicherheitshinweise finden Sie in den Sicherheitsdatenblättern (SDB) unter
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.
- In case any customization is required, please contact biotechrabbit via oem@biotechrabbit.com.

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Certified Quality-System mdc ISO 13485 ISO 9001

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