5X CAPITAL™ 1-Step qRT-PCR Probe Master Mix, lyophilized

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BC0503202	200 rxn of 20 µl	2 × Lyo CAPITAL qRT-PCR Probe Master Mix, 100 rxn 2 × 500 µl 5X qRT-PCR Probe Reconstitution Buffer

COMPONENT	COMPOSITION
Lyo CAPITAL qRT-PCR Probe Master Mix	Cake of 100 rxn lyophilized CAPITAL qRT-PCR Probe Master Mix
qRT-PCR Probe Reconstitution Buffer	Optimized 5X PCR buffer for reconstituting lyophilized CAPITAL qRT-PCR Probe Mix

LYO MASTER MIX RECONSTITUTION

1) Transfer 400 µl of the 5X qRT-PCR Mix Reconstitution Buffer to one vial Lyo CAPITAL qRT-PCR Probe Mix. Discard the remaining overfill.
2) Mix well – the lyophilisate will dissolve within seconds
3) Store the reconstituted CAPITAL qRT-PCR Probe Mix at -30°C to -10°C

STORAGE

Store at room temperature or below (until expiry date – see product label)
Reconstituted lyophilisate: store at -30°C to -10°C for up to 12 months

FEATURES

- Stable enzyme and mix for ambient shipment and room-temperature storage
- Best in-class performance for both single and multiplex detection
- Convenient master mix for detection of low-copy pathogen targets
- 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

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DESCRIPTION

biotechrabbit™ lyophilized CAPITAL 1-Step qRT-PCR Probe Master Mix is a freeze-dried version of the well-established liquid equivalent. The stabilized format allows shipment and storage without cooling. The master mix is optimized for real-time PCR quantification of RNA templates, including mRNA, total RNA and viral RNA from a wide range of targets. The mix ensures high specificity and sensitivity in single and multiplex detection, making it the choice for extremely low-copy-number targets in pathogen detection.
CAPITAL 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).

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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, DNases 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 CAPITAL 1-Step qRT-PCR Probe Master Mix (reconstituted lyophilizate)	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.

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CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Mix tested functionally in qRT-PCR.

Quality confirmed by: Head of Quality Control

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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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