

## ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0502901	500 rxns of 20 µl	1 ml 10X LYO-ready CAPITAL™ qRT-PCR Probe Master Mix 2 ml 5X qRT-PCR Probe Reconstitution Buffer
BR0502903	5000 rxns of 20 µl	10 ml 10X LYO-ready CAPITAL™ qRT-PCR Probe Master Mix 20 ml 5X qRT-PCR Probe Reconstitution Buffer
BR0503001	500 rxns of 20 µl	1 ml 10X LYO-ready CAPITAL™ qRT-PCR Probe Master Mix 5 ml 2X qRT-PCR Probe Reconstitution Buffer
BR0503003	5000 rxns of 20 µl	10 ml 10X LYO-ready CAPITAL™ qRT-PCR Probe Master Mix 50 ml 2X qRT-PCR Probe Reconstitution Buffer

COMPONENT	COMPOSITION
10X LYO-ready CAPITAL™ qRT-PCR Probe Master Mix	10X concentrated formulation of a qRT-PCR Probe Master Mix including excipients ready for lyophilization
2X or 5X qRT-PCR Probe Reconstitution Buffer	Optimized PCR reconstitution buffer in 2X or 5X concentration

## STORAGE

Store at –20 °C until expiry date.

## FEATURES

- Freeze-dryable formulation including excipients and stabilizers
- Best-in-class performance in both single and multiplex quantitative amplification
- Convenient master mix for low-copy number pathogen detection
- High specificity and sensitivity across a wide range of sample sources

## APPLICATIONS

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

## DESCRIPTION

biotechrabbit™ 10X LYO-ready CAPITAL™ qRT-PCR Probe Master Mix is a ready-to-use and concentrated formulation of all enzymes, dNTPs, excipients, and stabilizers for producing home-made lyophilizates with the option to include primers and probes. The time-effective lyophilization is possible for high and low volumes of the master mix without the collapse of the lyophilizate. The resulting lyophilizate is highly resistant to humidity and is swiftly dissolved in the provided reconstitution buffer. The reconstitution buffer is available in two different concentrations.

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 amplification, making it the best choice for extremely low-copy-number targets in pathogen detection.

CAPITAL™ qRT-PCR Probe Master Mix uses proprietary formulation for efficient RNA transcription and qPCR in a single tube.

## HANDLING

### Prevention of contamination

RNase contamination is a concern when handling RNA; to minimize the risk of contamination and prevent RNA degradation follow the guidelines below:

- Wear disposable gloves when handling the solutions.
- Dedicate separate sterile areas for the preparation of samples and reaction mixtures.
- Use molecular-grade nuclease-free water and reagents.
- Include a non-template control reaction in every PCR assay.
- Avoid carryover contamination.

### Before starting a lyophilization project, please consider the following recommendations

- The formulation is optimized for freeze-drying a minimum of 5 reactions of 20 µl.
- *Optional:* Prepare concentrated primers and probes in molecular biology grade water and restrict the volume to 20 % of the master mix.

## CALCULATION EXAMPLES

Three calculation examples for 20 µl reaction size

PARAMETER	EXAMPLE A	EXAMPLE B	EXAMPLE C
qRT-PCR volume	20 µl	20 µl	20 µl
Reactions per bead/cake	5	50	100
Number of beads/cakes	1	10	50
Total number of reactions	5	500	5000
Total reaction volume	100 µl	10 ml	100 ml

## PIPETTING SCHEME

Divide the total reaction volume above, by a factor of 10; minimize the volume of primers and probes

COMPONENT	EXAMPLE A	EXAMPLE B	EXAMPLE C
10X LYO-ready CAPITAL™ qRT-PCR Probe Master Mix	10 µl	1 ml	10 ml
<i>Optional:</i> Primer & Probes (max. 20 % v/v)	2 µl	200 µl	2 ml
Volume to freeze-dry per bead/cake	12 µl	120 µl	240 µl

## SUGGESTED LYOPHILIZATION PROTOCOL

STEP	TIME*	TEMPERATURE	PRESSURE
Freezing	120 min	-40 °C	---
Primary drying	600 min	-40 °C	0.12 mbar / 12 Pa
Secondary drying	300 min	25 °C	0.01 mbar / 1 Pa

\*Duration of freeze-drying cycles depends on the lyophilizate volume, the equipment, the type and dimensions of the vials.

**RECONSTITUTION OF LYOPHILIZED MASTER MIX**

- Add the required volume of the reconstitution buffer to the lyophilized CAPITAL™ qRT-PCR Probe Master Mix (see examples below)
- Mix well – the lyophilizate should dissolve swiftly
- Store the reconstituted CAPITAL™ qRT-PCR Probe Master Mix at –20 °C

PARAMETER	EXAMPLE A	EXAMPLE B	EXAMPLE C
2X Reconstitution Buffer per bead/cake	50 µl	500 µl	1 ml
5X Reconstitution Buffer per bead/cake	20 µl	200 µl	400 µl
Total volume of 2X Reconstitution Buffer	50 µl	5 ml	50 ml
Total volume of 5X Reconstitution Buffer	20 µl	2 ml	20 ml

**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 T<sub>m</sub> +2 °C or use gradient PCR to optimize the annealing temperature. Do not use annealing temperatures below 60 °C.

## QUALITY CONTROL ASSAYS

### Functional assay

The performance of the reconstituted mix was confirmed by qRT-PCR.

## SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)

Sicherheitshinweise finden Sie in den Sicherheitsdatenblättern (SDB) unter

<http://www.biotechrabbit.com/support/documentation.html>

## USEFUL HINTS

- Visit Applications at [www.biotechrabbit.com](http://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](mailto:oem@biotechrabbit.com).

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valid from 08.05.2023