CAPITAL™ qRT-PCR Green Mix, 4×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0502301	200 rxn of 20 µl	1 ml CAPITAL qPCR Green Mix (1step) 200 µl RTase with RNase Inhibitor
BR0502302	1000 rxn of 20 µl	5 × 1 ml CAPITAL qPCR Green Mix (1step) 5 × 200 µl RTase with RNase Inhibitor
BR0502401	200 rxn of 20 µl	1 ml CAPITAL qPCR Green Mix LRox (1step) 200 µl RTase with RNase Inhibitor
BR0502402	1000 rxn of 20 µl	5 × 1 ml CAPITAL qPCR Green Mix LRox (1step) 5 × 200 µl RTase with RNase Inhibitor
BR0502501	200 rxn of 20 µl	1 ml CAPITAL qPCR Green Mix HRox (1step) 200 µl RTase with RNase Inhibitor
BR0502502	1000 rxn of 20 µl	5 × 1 ml CAPITAL qPCR Green Mix HRox (1step) 5 × 200 µl RTase with RNase Inhibitor

COMPONENT	COMPOSITION
CAPITAL qPCR Green Mix (1step)	Optimized 4× qPCR Green Master Mix for One Step qRT-PCR containing proprietary Green dye
LRox Mix / HRox Mix	Rox incorporated in the mix in low / high concentration
RTase with RNase Inhibitor	Proprietary 20× Reverse transcriptase in a mix with efficient Ribonuclease Inhibitor
STORAGE	−20°C (until expiry date – see product label) Protect from light. Avoid multiple freeze thaw cycles by preparing aliquots.

FEATURES

- Convenient mix for quantification of RNA templates
- Sensitive and specific amplification with rapid extension rate for early Ct values
- Excellent linearity across a wide range of RNA dilution

APPLICATIONS

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

DESCRIPTION

biotechrabbit™ CAPITAL qRT-PCR Green Mix allows sensitive and specific cDNA synthesis and qPCR in a single tube for quantifying mRNA, total RNA and viral RNA sequences. Extremely low-copy-number targets can be detected with high efficiency over several logs of template concentration.
CAPITAL qRT-PCR Green Mix uses proprietary reverse transcriptase technology and buffer chemistry for efficient cDNA synthesis and QPCR in a single tube. To enable the use of the kit on qPCR platforms with different reference dye concentration requirements, three kit formats are available: a one-step kit containing no ROX, as well as LRox and HRox versions containing ROX in the corresponding concentrations.

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

ROX REFERENCE DYE

- See PCR cycler instruction for recommended concentration of ROX passive reference dye

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/).

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 qRT-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.		
Template RNA	Variable	0.01 pg to 1 µg
Use 1 pg – 1 µg Total RNA, or >0.01 pg mRNA		
CAPITAL qPCR Green Mix, 4×	5 µl	1×
RTase with RNase Inhibitor, 20×	1 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 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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valid from 09.11.2018