

4X CAPITAL™ qPCR Probe Master Mix

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0501401	200 reactions of 20 µl	1 ml CAPITAL qPCR Probe Master Mix
BR0501402	1000 reactions of 20 µl	5 × 1 ml CAPITAL qPCR Probe Master Mix
BR0501501	200 reactions of 20 µl	1 ml CAPITAL qPCR Probe Mix Master LROX
BR0501502	1000 reactions of 20 µl	5 × 1 ml CAPITAL qPCR Probe Master Mix LROX
BR0501601	200 reactions of 20 µl	1 ml CAPITAL qPCR Probe Master Mix HROX
BR0501602	1000 reactions of 20 µl	5 × 1 ml CAPITAL qPCR Probe Master Mix HROX

COMPONENT

COMPOSITION

CAPITAL qPCR Probe Master Mix

Optimized 4X qPCR Probe Master Mix

LROX Mix / HROX Mix

Rox incorporated in the mix in low / high concentration

STORAGE

-30°C to -10°C (until expiry date – see product label).

Avoid multiple freeze thaw cycles by preparing aliquots.

FEATURES

- Best in-class performance for both single and multiplex detection
- Convenient master mix with high specificity in pathogen detection
- Highly sensitive for low-abundance DNA targets

APPLICATIONS

- Standard and fast cycling qPCR with rapid extension rate for early Ct values
- For use on a wide range of probe technologies including Taqman®, Molecular Beacons® and Scorpion® probes

4X CAPITAL™ qPCR Probe Master Mix

DESCRIPTION

biotechrabbit™ 4X CAPITAL qPCR Probe Master Mix for quantifying genomic, cDNA and viral sequences provides outstanding performance in single and multiplex qPCR. The high sensitivity provided by the mix is ideal for detection of low-abundance DNA targets in various applications, such as pathogen detection.

CAPITAL qPCR Probe Mix uses proprietary combination of enzyme and buffer chemistry for efficient extension and early Ct in single and multiplex qPCR. To enable the use of the kit on qPCR platforms with different reference dye concentration requirements, three kit formats are available: a 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 T_m (use gradient PCR to optimize the annealing temperature).

ROX REFERENCE DYE

- See PCR cyclers 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.
- Amplicon lengths should not exceed 400 bp.
- 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 PCR contamination

When assembling the amplification reactions, care should be taken to eliminate the possibility of contamination with undesired DNA.

- Use separate clean areas for preparation of samples and reaction mixtures and for cycling.
- Wear fresh gloves. Use sterile tubes and pipette tips with aerosol filters for PCR setup.
- Use only water and reagents that are free of DNA and nucleases.
- With every PCR setup, perform a contamination control reaction that does not include 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
<i>Too high primer concentrations result in unspecific amplification and should be avoided.</i>		
Specific Probe	Variable	200 nM
Template DNA	Variable	10 pg – 100 ng
<i>Use diluted or undiluted cDNA from less than 1 µg RNA</i>		
4X CAPITAL qPCR Probe Master Mix	5 µl	1×
Nuclease free water	Variable	
Total volume	20 µl	
<ul style="list-style-type: none"> • Gently mix the reactions without creating bubbles (do not vortex). Bubbles will interfere with fluorescence detection. • Place the reaction into the PCR cyclor. 		

CYCLING PROGRAM

STEP	TEMPERATURE	TIME	CYCLES
Initial activation	95°C	2-3 min	1
Denaturation	95°C	10–15 s	40–45
Annealing/Extension*	(60–68°C)	30 s	

*Recommended annealing/extension temperature is primer $T_m + 2^\circ\text{C}$. Use gradient PCR to optimize the annealing temperature. Do not use temperatures below 60°C.

Do not exceed 30 seconds. For melt analysis refer to instrument instructions.

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CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Mix tested functionally in qPCR.

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.biotechrabbith.com/support/documentation.html>.

USEFUL HINTS

- Visit Applications at www.biotechrabbith.com for more products and product selection guides.
- Most biotechrabbith products are available in custom formulations and bulk amounts.

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