CAPITAL™ qPCR Probe Mix, lyophilized, 4×

biotechrabbit
leap and lead

LOT: See product label   EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0502601	200 rxn of 20 µl	2 × Lyo CAPITAL qPCR Probe Mix, 100 rxn 2 × 500 µl qPCR Probe Reconstitution Buffer

COMPONENT	COMPOSITION
Lyo CAPITAL qPCR Probe Mix	Cake of lyophilized 4× qPCR Probe Master Mix
qPCR Probe Reconstitution Buffer	Optimized PCR buffer for reconstituting lyophilized CAPITAL qPCR Probe Mix

LYO MASTER MIX RECONSTITUTION

1) Transfer the whole content of one vial qPCR Mix Reconstitution Buffer to one vial Lyo CAPITAL qPCR Probe Mix
2) Mix well – the lyophilisate will dissolve within seconds
3) Store the reconstituted CAPITAL qPCR Probe Mix at -20°C

STORAGE

Store at room temperature or below (until expiry date – see product label)
Reconstituted lyophilisate: store at −20°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 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

DESCRIPTION

biotechrabbit™ lyophilized CAPITAL qPCR Probe 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 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.

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.
- 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
Too high primer concentrations result in unspecific amplification and should be avoided.		
Specific Probe	Variable	200 nM
Template DNA	Variable	10 pg – 100 ng
Use diluted or undiluted cDNA from less than 1 µg RNA		
CAPITAL qPCR Probe Mix, 4× (reconstituted lyophilisate)	5 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
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 Tm +2°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.

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.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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