2X 5Star™ qPCR Probe Master Mix

Ordering Information

CAT. No.	Size	Package Content
BC0502501	100 rxns of 20 µl	1 ml 2X 5Star™ qPCR Probe Master Mix

Component	Composition
2X 5Star™ qPCR Probe Master Mix	Optimized and robust 2X 5Star™ qPCR Probe Master Mix
Storage	Store at −20 °C until expiry date.

Features

- Robust amplification in both single and multiplex quantitative PCR
- Suitable for amplification from crude lysates and detection of low-copy numbers
- High specificity and sensitivity across a wide range of sample sources
- Excellent performance in presence of PCR inhibitors
- Compatible with a wide range of quantitative PCR platforms

Applications

- Standard and fast cycling for both single and multiplex qPCR
- Suitable for routine and high-throughput laboratories
- Compatible with a wide range of probe technologies including Taqman®, Molecular Beacons® and Scorpion® probes
- For gene expression analysis, genotyping, pathogen detection and quantification

Description

biotechrabbit™ 2X 5Star™ qPCR Probe Master Mix is an antibody-mediated hot-start real-time PCR mix optimized for amplification and quantification of viral DNA, cDNA and genomic DNA from a wide range of targets. The mix is tailored for high specificity and sensitivity in single and multiplex amplification; 5Star™ qPCR Mix is an excellent choice for the amplification of crude lysates, templates carrying PCR inhibitors and targets with low copy numbers.
5Star™ qPCR Probe Master Mix uses a proprietary enzyme and buffer formulation suitable for fast extension and multiplexing in challenging PCR reactions.

ROX REFERENCE DYE

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

Notes

- For efficient amplification under fast cycling conditions use amplicon lengths between 80 bp and 200 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.

HANDLING

Prevention of contamination

Contamination with undesired DNA is a concern when assembling the amplification reactions. To eliminate the possibility of contamination with undesired DNA, 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.

BASIC PROTOCOL

- Calculate the required volume for your desired reaction numbers and consider one additional.
- Use high-quality PCR plates and seals designed for fluorescence applications.
- Reserve plate positions for positive (control DNA) and negative (water or buffer) controls.
- For 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 to 100 ng
Use diluted or undiluted cDNA from less than 1 µg RNA		
2X 5Star™ qPCR Probe Master Mix	10 µl	1X
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	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.

QUALITY CONTROL ASSAYS

Functional assay

Miix tested functionally in qPCR.

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 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.

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