BioChain®
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User's Manual and Instructions

Pro QPCR SuperMix Kit

Catalog Number: K5053200, K5053400

Introduction

Real–time or quantitative PCR (QPCR) allows quantification of DNA, cDNA, or RNA templates. QPCR is based on the detection of a fluorescent reporter molecule that increases as PCR products accumulate with each cycle of amplification. The use of fluorescent probe technologies reduces the risk of sample contamination while maintaining convenience, speed and high throughput screening capabilities. BioChain's Pro QPCR SuperMix can be used with fluorescent probe technologies to perform real-time PCR. The Pro QPCR SuperMix is a ready-to-use, 2x- concentrated master mix that contains all the reagents (except primers, probe and templates) needed for running quantitative, real-time DNA detection assays, in the fluorescent probe-based detection assays such as TaqMan® or molecular beacons. The passive reference dye ROX is included in the Pro QPCR SuperMix for many real-time QPCR platforms.

BioChain's QPCR SuperMix contains BioChain's Taq DNA polymerase with hot start capability. BioChain's hot-start Taq DNA polymerase improves PCR amplification reactions by decreasing non-specific amplification and preventing primer-dimer formation. This enzyme is activated after an initial seven to ten minutes heating at 95°C. And the real-time PCR buffer is specially formulated to provide superior specificity and increase amplification efficiency. This SuperMix can amplify and detect a broad range of DNAs or cDNAs, including those GC- or AT-rich targets.

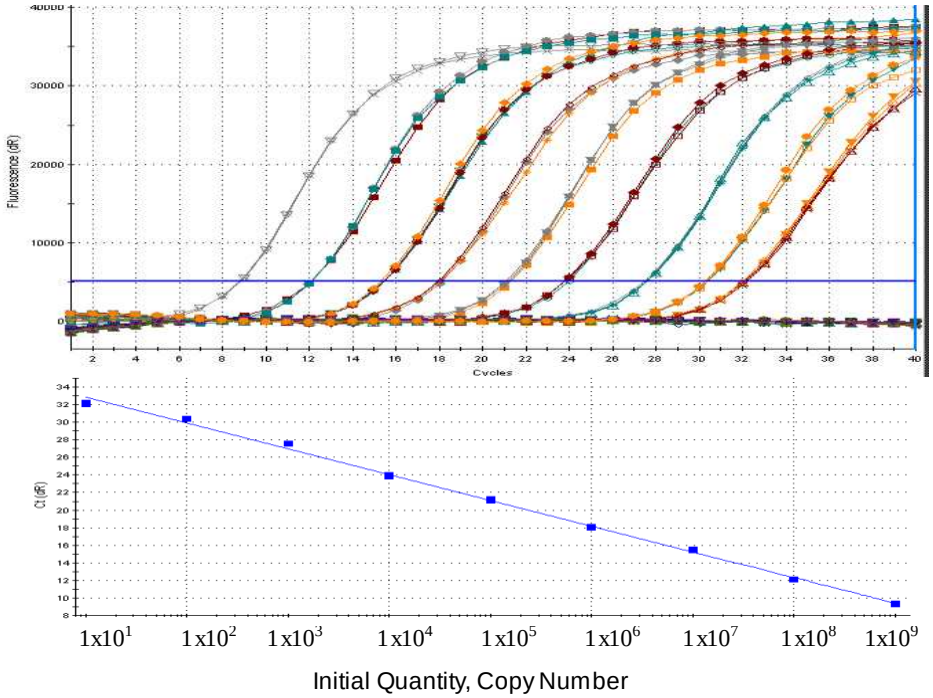

Figure 1. BioChain Pro QPCR SuperMix amplifies over a broad dynamic range. 10 to 1 x 10^9 copies of plasmid containing cDNA of human GAPDH gene were amplified in 25 µl reactions. Highly reproducible triplicates demonstrated good linearity of 0.997 and excellent PCR efficiency of 102.1% over a 9-order of dynamic range. BioChain's Pro QPCR SuperMix has high sensitivity, detecting as few as 10 copies of target DNA within the linear range.

Features

- Convenient - All reaction components are supplied for quick and easy set up
- Save time - Ready-to-use SuperMix reduces setup time and liquid handling steps
- Wide dynamic range: good linearity and excellent PCR efficiency over an 9 orders of dynamic range
- High Sensitivity - detect as low as 10 copies of DNA.
- Amplify and detect a broad range of DNA or cDNA targets- including those that are GC- or AT-rich
- Flexible – Compatible with most of the real-time PCR instruments.

Applications

- Real-Time PCR
- Gene expression profiling
- Gene knockdown verification
- Array validation

Description

Components in this kit are prepared with pure chemicals according to our proprietary technology. BioChain's Pro QPCR SuperMix is a 2x concentration of premix reagent including Hotstart DNA polymerase and specially formulated real time buffer designed for real-time PCR with fluorescent probe-based detection format.

Quality Control

1 kit of this lot has been tested for amplifying plasmid containing human GAPDH cDNA (amplified fragment: 77 bp) over a 9 orders of dynamic range using Stratagene's Mx3005P as a real time PCR instrument. Good linearity and great PCR efficiency is observed and consistent with the previous lot.

Components

Pro QPCR SuperMix Kit:

Catalog Number: K5053200: Reagents are sufficient for 200 25 μl volume assays

Item	Amount	Part No.
1. Pro QPCR SuperMix	1.25 ml x 2	K5053200-1

Catalog Number: K5053400: Reagents are sufficient for 400 25 μl volume assays

Item	Amount	Part No.
1. Pro QPCR SuperMix	1.25 ml x 4	K5053400-1

Reagents and Equipments Required but not Supplied in this Kit:

1. Nuclease-free PCR-grade water
2. Spectrofluorometric thermal cycler

Storage and Stability

Upon receipt, store all components at 4 ºC in a constant temperature refrigerator. When stored under these conditions the supermix is stable for one year after ship date.

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Protocol

(Using Stratagene's Mx3000P™/Mx4000®, and ABI PRISM®/GENEAmp® 5700 Real-time PCR Instrument)

Reagent Preparation and Storage

Pro QPCR SuperMix is ready to use and stored at 4ºC for and ready use. Avoid direct light in preparation of the PCR reaction mixture because probe and ROX reference dye are light sensitive.

1. If the ROX reference dye will be included in the reaction, keep all solutions containing the ROX protected from light.

2. (Optional) Set up a no-template control to screen for contamination of reagents or false amplification.

3. Due to the sensitivity of quantitative PCR, results can be easily affected by pipetting errors. Always prepare a master mix of Pro QPCR SuperMix containing the primers, probe. Individual pipetting of replicate samples is not recommended.

Real-time PCR Cycling Programs

4. Prepare the following PCR reaction mixture. (First make the master mix without the template. After making the master mix, gently mix the reaction without creating the bubbles, aliquot and then add 2 μl of template to each experimental reaction)

per reaction: 25 μl

Regents	Volume	Final Concentration
Pro QPCR SuperMix (2x)	12.5 μl	1x
PCR forward primer	X μl	200-500 nM
PCR reverse primer	X μl	200-500 nM
PCR probe	X μl	100-500 nM
Template [b]	2 μl	
Nuclease-free PCR grade water	Add up to 25 μl	

[b] Final template concentration varies depending on the copy number of target present in the template solution. Optimal amount should be determined by preparing the dilution series. It is recommended to use DNA template in less than 100 ng.

5. Gently mix the reactions without creating bubbles since bubbles interfere with fluorescence detection. Then centrifuge the reactions briefly.

6. Place the reactions in the instrument and run the appropriate PCR program. Try the following protocol firstly, and optimize the reaction condition if needed.

PCR program for amplification:

Cycles	Temperature	Time	Detection	Remark
1	95°C	7-10 min.	OFF	This step will activate the Hotstart DNA polymerase.
40	95°C	30 sec	OFF	Set the instrument to detect and report fluorescence either
	55-65°C [a]	1 min	ON	

	72°C	30 sec to 1.5 min[b]	OFF	at the annealing step or the extension step of each cycle.
1	72°C	3 min	OFF	This step can be omitted if the amplicon size is <300 bp.

a. Set an appropriate annealing temperature for the primer set used.
b. Set the extension time to 1-1.5 min if the amplicon size is > 400 bp.

Related Products
Eva QPCR SuperMix (Cat# K5053200, K5053400), dNTP set for PCR (Cat# K6011100), PCR mix (Cat# 5051100), PCR Optimization Kit (Cat# K5051100), Taq Polymerase (Cat# 7051200), RNA, PCR ready cDNA, and PCR ready genomic DNA.

References

1. Higuchi R, Dollinger G, Walsh P S and Griffith R (1992): Simultaneous amplification and detection of specific DNA sequences. *BioTechnology* 10:413-417.

2. Higuchi R, Fockler C, Dollinger G and Watson R (1993): Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *BioTechnology* 11:1026-1030

3. Bustin, S A (2000): Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. Journal of Molecular Endocrinology 25:169-193.