BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

Fast Pro QPCR SuperMix Kit – ROX premixed

Catalog Number: K5058200, K5058400

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 nonspecific products while maintaining convenience, speed and high throughput screening capabilities. The Fast 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 utilizing fluorescent probe-based technologies such as TaqMan® or molecular beacons. The passive reference dye ROX is premixed in the Fast Pro QPCR SuperMix – ROX premixed.

BioChain's QPCR SuperMix contains BioChain's Taq DNA polymerase. 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.

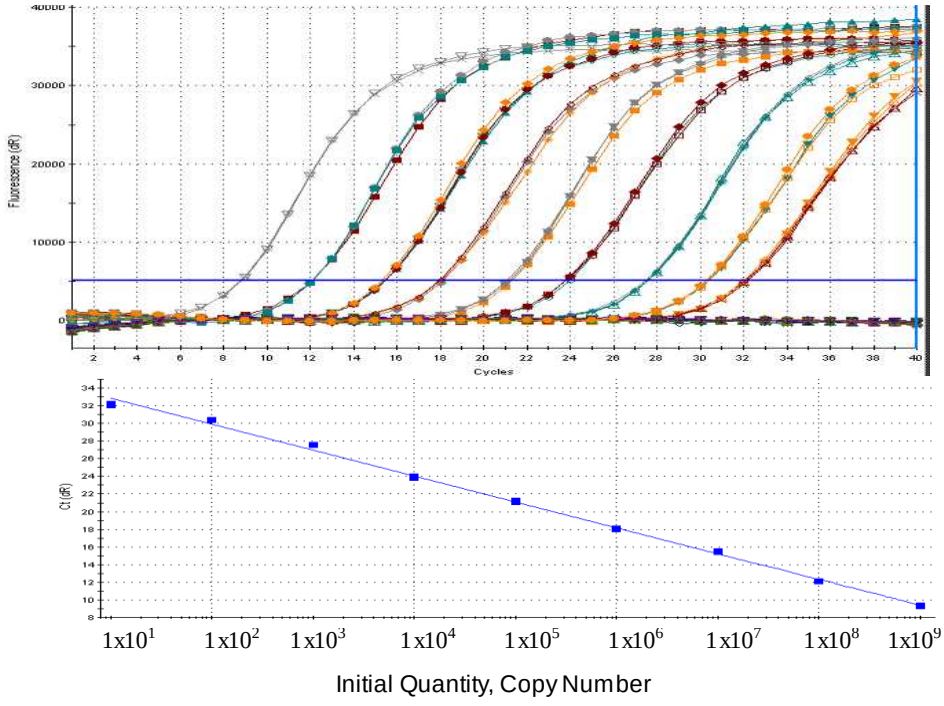

Figure 1. BioChain Pro QPCR SuperMix amplifies over a broad dynamic range. 10 to 1 x 10^9 copies of plasmid containing cDNA of human GAPDH gene were amplified in 25 µl reactions. Highly reproducible triplicates demonstrated good linearity of 0.997 and excellent PCR efficiency of 102.1% over a 9-order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

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

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 Fast Pro QPCR SuperMix – ROX premixed is a 2x concentration of qPCR reagent including Taq DNA polymerase and a 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

Fast Pro QPCR SuperMix Kit – ROX premixed:

Catalog Number: K5058200: Reagents are sufficient for 200 25 μl volume assays

Item	Amount	Part No.
1. Fast Pro QPCR SuperMix – ROX premixed	1.25 ml x 2	K5058200-1

Catalog Number: K5058400: Reagents are sufficient for 400 25 μl volume assays

Item	Amount	Part No.
1. Fast Pro QPCR SuperMix – ROX premixed	1.25 ml x 4	K5058400-1

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 -20ºC in a constant temperature freezer. When stored under these conditions the supermix is stable for one year after ship date.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

Protocol

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

Reagent Preparation and Storage

Fast Pro QPCR SuperMix – ROX premixed is stored at -20ºC. Fully thaw and mix the Fast Pro QPCR Supermix before use. Avoid direct light in preparation of the PCR reaction mixture due to the light sensitivity of the probe and ROX dye.

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

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

Real-time PCR Cycling Programs

3. The following is a sample PCR reaction setup. (First make the master mix components without the template. After making the master mix, gently mix the reagents without creating bubbles, aliquot and then add 2 µl of template to each experimental reaction)

per reaction: 25 µl

Regents	Volume	Final Concentration
Fast Pro QPCR SuperMix – ROX premixed (2x)	12.5 µl	1x
PCR forward primer	X µl	200-800 nM
PCR reverse primer	X µl	200-800 nM
PCR probe	X µl	100-500 nM
Template [a]	2 µl	
Nuclease-free PCR grade water	Add up to 25 µl	

[a] *Final template concentration varies depending on the copy number of target present in the sample. The optimal amount should be determined by preparing a dilution series. It is recommended to keep the quantity of DNA used below 100ng.*

4. After aliquoting the sample and PCR buffer into the proper wells, briefly centrifuge the samples down.

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

   PCR program for amplification:

Cycles	Temperature	Time	Detection	Remark
1	95°C	1 min.	OFF	
40	95°C	5 sec	OFF	Set the instrument to detect and report fluorescence at the annealing/extension step of each cycle.
	60°C	30s	ON	
1	72°C	3 min	OFF	This step can be omitted if the amplicon size is <300 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.