User's Manual and Instructions

Product: Eva QPCR SuperMix Kit

Catalog Number: K5052200, K5052400

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. In BioChain's Eva QPCR SuperMix, a superior green fluorescence DNA-binding dye is used for real-time detection and quantitation of DNA. The Eva QPCR SuperMix is a ready-to-use, 2x-concentrated master mix that contains all the reagents (except primers and templates) needed for running quantitative, real-time DNA detection assays, in the double-stranded DNA dye detection format. The passive reference dye ROX is included in a separate tube to make the Eva Supermix adaptable for many real-time QPCR platforms. A pair of human beta-actin primers is also included in the kit as a control.

BioChain's QPCR SuperMix contains BioChain's Taq polymerase with hot start capability. BioChain's hot-start Taq 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 DNA or cDNA targets, including those are GC- or AT-rich.

Eva Dye

Eva Dye binds double–stranded DNA. Detection is monitored by measuring the increase in fluorescence intensity throughout the cycle. Eva Dye has higher affinity to double-stranded DNA than SYBR Green dye and shows stronger fluorescence intensity than SYBR Green upon binding to DNA. Eva Dye is more stable than SYBR Green and the absorption and emission spectra of Eva Dye are similar to SYBR Green Dye or FAM, so the same optical setting for SYBR Green Dye or FAM can also be used for Eva Dye.

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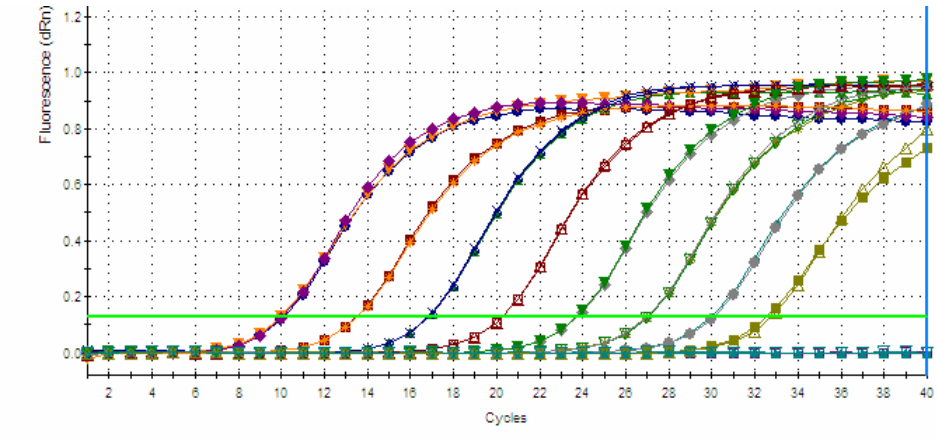
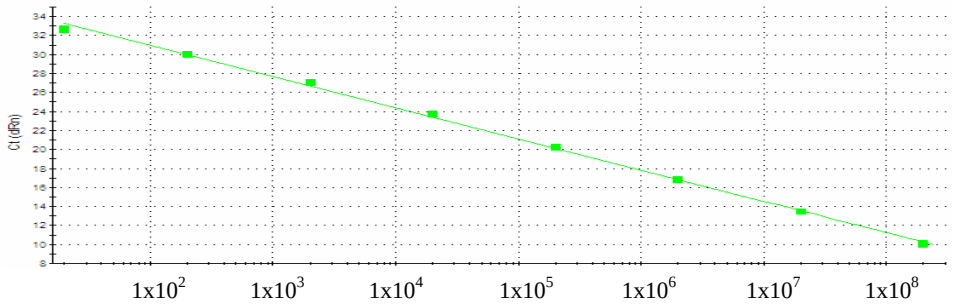


Y=-3.289Log(x)+37.56, R^2=0.999, Efficiency=101.4%



Initial Quantity, Copy Number

Figure 1. BioChain Eva QPCR SuperMix amplifies over a broad dynamic range. 2 x 10^1 to 2 x 10^8 copies of plasmid containing cDNA of human beta-actin gene were amplified in 25 µl reactions. Highly reproducible triplicates demonstrated good linearity of 0.999 and excellent PCR efficiency of 101.4% over an 8-order of dynamic range. BioChain's Eva SuperMix has high sensitivity, detecting as few as 20 copies of target DNA within the linear range.

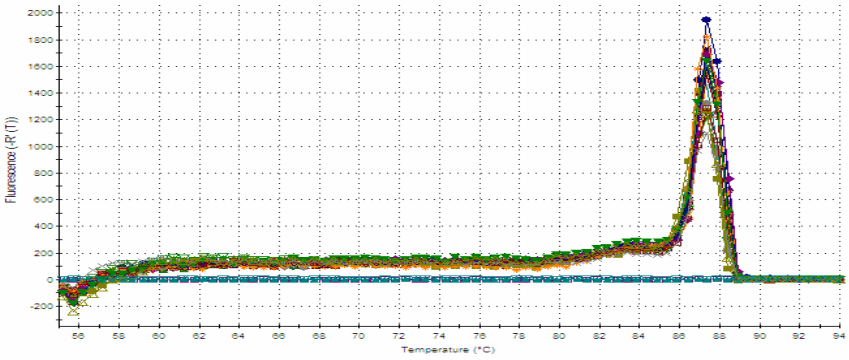


Figure 2. Dissociation Curve of PCR products amplifies over a broad dynamic range. *2 x 10^1 to 2 x 10^8 copies of plasmid containing cDNA of human β-actin gene were amplified in 25-µl reactions.*

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 8 orders of dynamic range
- High Sensitivity - detect as low as 20 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 QPCR SuperMix is a 2x concentration of premix reagent including Hotstart DNA polymerase and Eva Dye and specially formulated real time buffer designed for real-time PCR with intercalator format.

Quality Control

1 kit of this lot has been tested for amplifying plasmid containing human β-actin cDNA (amplified fragment: 202 bp) over an 8 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

Eva QPCR SuperMix Kit:

Catalog Number: K5052200: Reagents are sufficient for 200 assays

Item	Amount	Part No.
1. Eva QPCR SuperMix	1.25 ml x 2	K5052200-1
2. ROX reference Dye	50 µl x 2	K5052200-2
3. human β-actin control F/R primer pair (25x)	50 µl	K5052200-3

Catalog Number: K5052400: Reagents are sufficient for 400 assays

Item	Amount	Part No.
1. Eva QPCR SuperMix	1.25 ml x 4	K5052400-1
2. ROX reference Dye	50 µl x 4	K5052400-2
3. human β-actin control F/R primer pair (25x)	50 µl	K5052400-3

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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 -20 ºC in a constant temperature freezer. Avoid repeated freeze/thaw cycles. When stored under these conditions the supermix is stable for one year after ship date. You may aliquot the supermix and store a portion at 4°C for ready use. The thawed Eva QPCR SuperMix is stable at least for 3 months at 4ºC. The Eva Dye and the ROX reference dye are light sensitive and should be kept away from light whenever possible.

Protocol

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

Use of the ROX Reference Dye

ROX reference dye is included in this kit and may be added to compensate for non-PCR related variations in fluorescence. Addition of the reference dye is optional. Optimizing the ROX dye concentration within the qPCR reaction is an important aspect of setup. Too much ROX in the qPCR reaction will reduce background but also makes a low target signal difficult to distinguish from background. Conversely, too little ROX can increase background, meaning that low or weak target signals can be lost. For instruments that allow excitation at ~584 nm (such as Stratagene's Mx instrument and ABI 7500), firstly 1:10 dilute the ROX reference dye provided in the kit, then begin optimization using 0.5 μl **diluted** ROX reference dye in 25 μl qRT-PCR reaction. For instruments that do not allow excitation near 584 nm (such as ABI PRISM®/GENEAmp® 5700 instrument), begin optimization using 0.5 μl **undiluted** ROX reference dye in 25 μl qRT-PCR reaction.

Reagent Preparation and Storage

Thaw the tube containing Eva QPCR SuperMix on ice and store it on ice while setting up the reactions. After initial thawing, aliquot the supermix and store a portion at 4ºC for ready use. Avoid direct light in preparation of the PCR reaction mixture because Eva Dye is 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 Eva SuperMix containing the primers and the reference dye (if reference dye is used). 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
Eva QPCR SuperMix (2x)	12.5 μl	1x
PCR forward primer	X μl	100-200 nM
PCR reverse primer	X μl	100-200 nM
Reference Dye ROX [a]	0.5 μl	
Template [b]	2 μl	
Nuclease-free PCR grade water	Add up to 25 μl	

[a] See page 5: Use of the ROX Reference Dye
[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 Taq polymerase.
40	95°C	30 sec	OFF	Set the instrument to detect and report fluorescence either at the annealing step or the extension step of each cycle.
	55-65°C [a]	1 min	ON	
	72°C	30 sec to 1.5 min[b]	OFF	
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.

2. Dissociation Program for all PCR products
   Follow manufacturer's guidelines for setting up dissociation depending on the instrument's software version.

PCR Setup and Cycling Program for human β-actin control primer set (amplicon size = 202 bp)

1. 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
Eva QPCR SuperMix (2x)	12.5 μl
Human β-actin primer set (25x)	1 μl
Reference Dye ROX [a]	0.5 μl
Template [b]	2 μl
Nuclease-free PCR grade water	Add up to 25 μl

[a] See page 5: Use of the ROX Reference Dye
[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

2. PCR program for amplification of human β-actin amplicon.

Cycles	Temperature	Time	Detection

1	95°C	10 min.	OFF
40	95°C	30 sec	OFF
	55-65°C	1 min	ON
	72°C	30 sec	OFF

3. Dissociation Program: Follow manufacturer's guidelines for setting up dissociation depending on the instrument's software version.

Related Products

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

References

1. Biotium, Inc. at http://www.biotium.com/product/product_info/allcolor.pdf

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

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

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