All-in-One™ qPCR Mix

For universal quantitative real-time PCR

Cat. No. AOPR-0200 (200 qPCR reactions)
Cat. No. AOPR-0600 (600 qPCR reactions)
Cat. No. AOPR-1000 (1000 qPCR reactions)
Cat. No. AOPR-1200 (1200 qPCR reactions)
Cat. No. AOPR-4000 (4000 qPCR reactions)

Performance optimized for All-In-One™ qPCR Primers, All-In-One™ miRNA qPCR Primers, miProfile™ miRNA qPCR Arrays, ExProfile™ Gene qPCR Arrays, All-In-One™ First-Strand cDNA Synthesis Kit and All-In-One™ miRNA First-Strand cDNA Synthesis Kit

User Manual

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USER MANUAL

All-in-One™ qPCR Mix

I. Description

The All-in-One™ qPCR Mix provides fast and efficient SYBR® Green-based real-time quantitative PCR. The qPCR Mix uses a high-fidelity hot-start DNA polymerase, optimized reaction buffer and high-quality dNTPs to enable specific and sensitive amplification of even low-copy genes or miRNAs. The All-in-One™ qPCR Mix reduces experimental design time by providing a universal reaction condition that can be used with almost all primers and most real-time PCR instruments.

II. Related Products

GeneCopoeia offers comprehensive solutions for studying gene expression. A careful process of co-development ensures that they work well together and provide robust and reproducible results.

Product	Description
All-in-One™ First-Strand cDNA Synthesis Kit	Reverse transcribe mRNA into first –stand cDNA
All-in-One™ qPCR Primers	Validated, gene-specific primers ensure specificity and sensitivity (human, mouse and rat)
ExProfile™ Gene qPCR Arrays	High-throughput or focused group profiling of gene expression
All-in-One™ miRNA First-Strand cDNA Synthesis Kit	Reverse transcribe miRNA into first –stand cDNA
All-in-One™ miRNA qRT-PCR Detection Kits	SYBR® Green-based detection kit accurately quantifies miRNA expression
All-in-One™ miRNA qPCR Primers	Validated human,mouse,rat miRNA primers for robust,reproducible and reliable quantitation of miRNA activity
miProfile™ miRNA qPCR Arrays	High-throughput or focused group profiling of miRNA expression
RNAzol® RT RNA Isolation Reagent	Easy isolation of mRNA, microRNA or total RNA

III. Contents and Storage

Contents and storage recommendations for the All-in-One™ qPCR Mix are provided in the following table.

Cat. Nos. AOPR-0200, AOPR-0600, AOPR-1000, and AOPR-4000

Contents	Quantity	Storage temperature/ conditions
2×All-in-One™ qPCR Mix	2×1 ml 3×(2×1 ml) 5×(2×1 ml) 20×(2×1 ml)	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at –80°C in aliquots. Avoid repeated freezing/ thawing.
ROX Reference Dye (30μM)	1×80 μl 3×80 μl 5×80 μl 20×80 μl	–20°C (Stable for at least 12 months) Alternatively, the solution can also be stored at –80 °C in aliquots. Avoid repeated freezing/ thawing.

IV. Preparation

Wearing a lab coat, disposable gloves and protective goggles are recommended when handling chemicals.

IMPORTANT NOTES:

1. When using the All-One-qPCR Mix with miProfile miRNA qPCR Arrays and All-in-One miRNA First-Strand cDNA Synthesis Kit for miRNA expression profiling, please follow the miProfile miRNA qPCR array user manual for the complete instruction.
2. Store the kit at –20°C. Avoid storage or leaving reagents at 4°C or room temperature. Avoid light exposure at all times.
3. Mix reagents thoroughly by gently inverting tubes several times avoiding bubbles and then briefly centrifuge before use.
4. Prepare the reaction mix with PCR grade water.
5. Strictly follow standard procedures for PCR to avoid nucleic acid contamination and non-specific amplification.
6. Read all procedures before setting up the PCR reaction

V. Procedure

1. **Thaw the 2×All-in-One™ qPCR Mix and ROX Reference Dye as needed.**
2. **Prepare the PCR reaction mix on ice. See the example below.**

Reagent	Volume	Final concentration
2×All-in-One™ qPCR Mix [a]	10 µl	1×
PCR forward primer (2 µM) [b]	2 µl	0.2 µM [c]
PCR reverse primer (2 µM)	2 µl	0.2 µM
Template [d]	2 µl	
ROX Reference Dye [e](30µM) if needed	0.4 -0.1µl	600nM-150nM
Water (double distilled)		
▪ Not using ROX Reference Dye	4 µl	
▪ Using ROX Reference Dye	3.6-3.9µl	
Total	**20 µl**	

a. Use the 2×All-in-One™ qPCR Mix as half of the total reaction volume and adjust other reagents accordingly. If the total reaction volume is changed, maintain each component in the proper proportion.

b. Primers are important considerations to ensure success with real-time PCR. All-in-One™ human, mouse and rat primer sets from GeneCopoeia have been validated to provide specific and sensitive amplification even with low copy number genes. For designing your own primers, you may wish to use Oligo primer analysis software (Molecular Biology Insights) or Primer Premier software (Premier Biosoft International).

c. Primer concentration should be in the range of 0.2 to 0.6 µM. In general, a PCR reaction using 0.2 µM primers produces good results. If the PCR efficiency is low, consider increasing primer concentration. However, keep in mind that non-specific PCR products may also increase with increased primer concentration.

d. Generally, the amount of DNA template should be less than 100 ng. Because different templates contain varying copies of a target gene, it may be necessary to perform a gradient dilution to determine the optimal amount of DNA template to use. If reverse transcript cDNA is used as template, dilute before use. Do not add more than 5% of the original cDNA solution volume to the total qPCR reaction solution.

e. ROX Reference Dye is added only for qPCR instruments that require ROX for calibration. ROX Reference Dye provides an internal reference to which the reporter-dye signal can be normalized during data analysis. Normalization is necessary to correct for fluorescence fluctuations due to changes in concentration or volume. Adjust the ROX Reference Dye to optimal concentration according to different qPCR instruments.

Instrument	ROX per 20μl PCR Reaction	Final Concentration
BioRad iCycler，MyiQ，iQ5，CFX-96，CFX-384，Eppendorf Mastercycler realplex，Roche LightCycler 480，LightCycler 2.0	None	No ROX
ABI PRISM 7000/7300/7700/7900HT and 7900HTFast, ABI Step One, ABI Step One Plus	0.4 μl（0.2-0.4μl）	600 nM（300-600nM）
ABI 7500，7500 Fast, ABI Viia7， Stratagene Mx3000P， Mx3005P, Mx4000，	0.1 μl（0.02-0.1μl）	150 nM（30-150nM）

For other instruments which need calibration of ROX but have not been listed out in the table, please optimize the concentration of ROX according to the guide line of specific instrument.

3. **Mix the PCR reaction mix sufficiently and add to the PCR reaction tubes.**
4. **Briefly centrifuge to make sure all the reagents are at the bottom of the reaction tubes.**
5. **The following three-step method for programming the PCR reaction is recommended:**

Cycles	Steps	Temperature	Time	Detection
1	Initial denaturation	95°C	10 min	No
40	Denaturation	95°C	10 sec	No
	Annealing	55°C~60°C	20 sec	No
	Extension	72°C	15 sec	Yes

Notes

i. When using SYBR Green dye to monitor the qPCR reaction, a melting curve analysis should be performed immediately at the end of cycling. (example adapted from the iQ5 real-time PCR detection system from Bio-Rad):

Temperature range	Heating rate	Constant temperature	Detection
72–95°C	0.5°C/unit time	6 sec/unit time	Yes
25°C		30 sec	No

The conditions for your instrument may differ, consult the documentation of your qPCR instrument for instructions.

ii. The DNA polymerase used in the 2×All-in-One™ qPCR Mix is a special chemically modified hot-start enzyme. Incubation for 10 minutes at 95°C will sufficiently activate the enzyme.

iii. The actual annealing temperature should be adjusted around the primer melting temperature ranging from 55°C~60°C. However, the optimal annealing temperature may be outside of this range. Adjust the temperature according to actual reaction conditions

iv. The optimal fragment length to use for amplification during real-time PCR is in the range of 80-150bp.However, fragment lengths up to 300bp are possible.

v. The main condition for the above reaction are referred to in the iQ5 qPCR instrument manual from Bio-Rad.If a qPCR instrument from another commercial source is used, please reference the instrument manual and adjust the extention time and melting curve conditions accordingly.

VI. Example

Objective: The amplification efficiency and detection sensitivity of the 2×All-in-One™ qPCR Mix are assessed by standard curves made by gradient dilution of plasmid DNA. The target fragment is 102 bp.

Equipment: iQ5 instrument (Bio-Rad Laboratories)

Procedure:

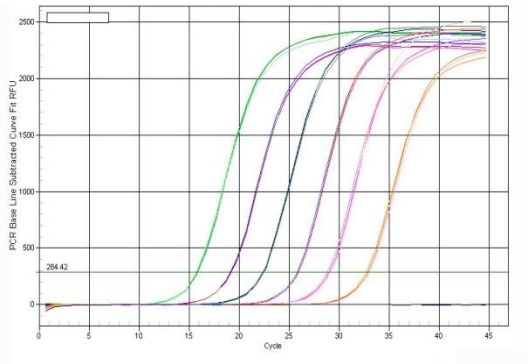
1. The plasmid is serially diluted to 6 concentrations ranging from 10^5 to 1 molecule/μl.
2. PCR reaction mix preparation (on ice)

Reagent components	**Volume**
2×All-in-One qPCR Mix	10 μl
PCR forward primer (2 μM)	2 μl
PCR reverse primer (2 μM)	2 μl
ddH_20	1 μl
Total	**15 μl**

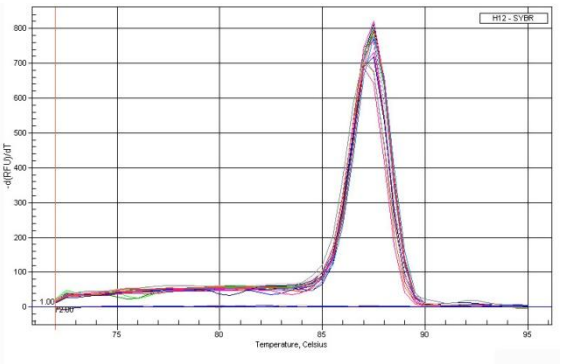
3. Mix the above reagents sufficiently. Aliquot to PCR tubes after a brief centrifugation.
4. Add 5 μl of the diluted plasmid template to each PCR tube. Use 5μl ddH_2O as a negative control.
5. Program the PCR reaction and corresponding reading conditions of the melting curve:

Cycles	**Steps**	**Temperature**	**Time**	**Detection**
1	Initial denaturation	95°C	10 min	No
45	Denaturation	95°C	10 sec	No
	Annealing	60°C	20 sec	No
	Extension	72°C	15 sec	Yes
	Melting curve reading	72°C~95°C	**Heating Rate** 0.5°C / 6 sec	Yes
	Cooling	25°C	30 sec	No

6. Analyze the amplification and corresponding melting curves after the qPCR experiment:

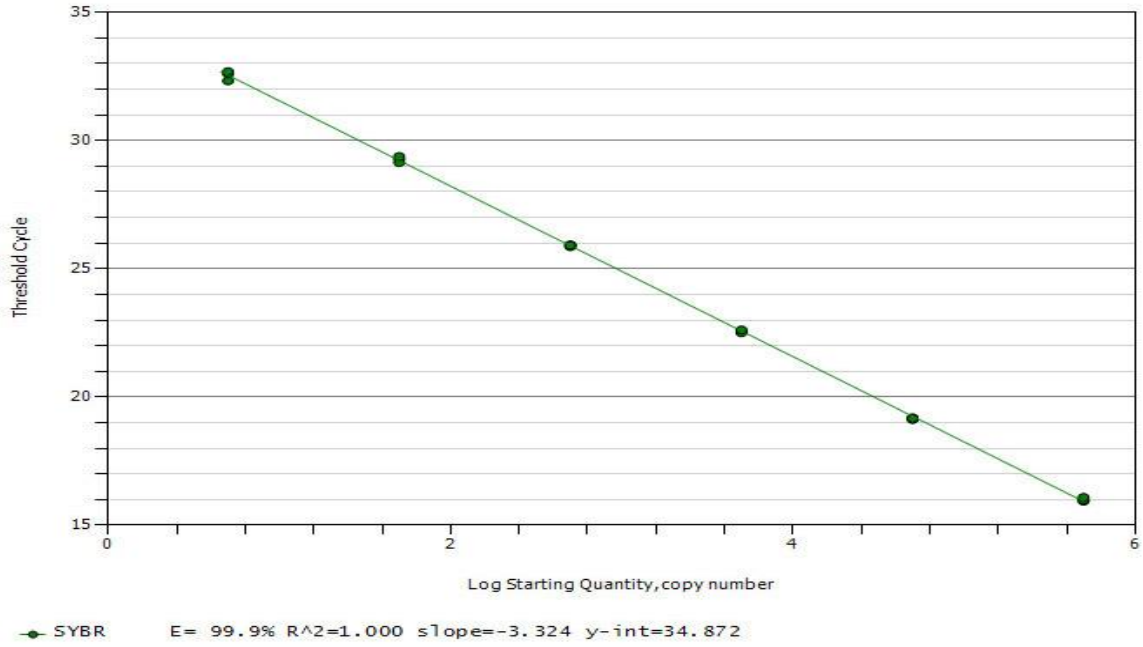


Amplification curves of serially diluted plasmid DNA



Peak values of amplified products in melting curves.

7. Construct a standard curve using the Ct values from each amplification curve:



Picture of a standard curve

8. **Conclusion**: The peak values from the amplification and melting curves show that as low as 5 molecules can be detected when using plasmid DNA as a template and that there is only a single amplified product, showing that very high sensitivity can be attained using the All-in-One™ qPCR Mix. At the same time, high amplification efficiency is also shown by the good linear relationship among each concentration of serially diluted plasmid.

VII. Trouble Shooting Guide

Poor precision or failed qPCR reactions	• Make sure the initial denature time was set as 10min, sufficiently activating of the hot-start polymerase could avoid non-specific amplification and production of primer-dimers. • The fluorescence detection temperature may not be appropriate. Adjust accordingly. • The set up position for reaction samples in the real-time PCR instrument may not be right. Adjust accordingly. • PCR cycle conditions, primer concentration and primer sequences may not be appropriate.Adjust the primer concentration and annealing temperature. If this does not work, redesign the primers. • The template sample purity may not be adequate. Purify the template sample by phenol/chloroform extraction and ethanol precipitation. If the samples are reverse transcribed cDNA, set up the qPCR reaction with a diluted sample as other concentrated reagents in the RT reaction mixture may be interfering with the qPCR. • Try to use 3.0% agarose gel electrophoresis to check the qPCR products. Check the purity of the primers by electrophoresis or use PAGE-purified primers if the bands are diffused. One may also use phenol/chloroform extraction and ethanol precipitation methods to treat the primers before the experiment.
Abnormal melting curves	**Signal in the blank (No Template Control) sample** • There may be contamination of the positive samples in the qPCR reaction system if the T_m of the melting curve of the blank control is the same as the positive control. Eliminate sample application error first. If the situation still persists, replace the PCR grade water and/or primers and/or use a new 2×All-in-One™ qPCR Mix. • If the T_m of the melting curve of the blank control is lower than the positive control, the qPCR reaction may have produced nonspecific amplification such as primer-dimers. Prepare the qPCR reaction mix on ice and increase the temperature of fluorescence detection. If this does not work, redesign the primers.
	Double peaks and multiple peaks in the melting curve of the positive control • In the absence of other primers present in the reaction, double or multiple peaks in the melting curve of the positive control indicate that the qPCR reaction produced nonspecific amplification fragments.

	Prepare the qPCR reaction mix on ice; optimize the qPCR reaction conditions, for example, by increasing the annealing temperature, decreasing the primer concentration or increasing the fluorescence detection temperature (not more than the T_m value of the expected product). If this does not work, redesign the forward primer. **No peaks or abnormal peaks in the melting curve(or the amplification curves) of the positive control** • Adjust the ROX Dye to optimized concentration according to instrument.
No signal (Ct) or late appearing signal	• Not enough PCR cycles. For good sensitivity, one should generally set up more than 35 PCR cycles, but more than 45 cycles may result in too much background signal. • The amount of template used may not be enough or the template may be degraded. Use the highest concentration possible of diluted template samples to set up the qPCR. At the same time, avoid freezing and thawing the samples repeatedly. • The amplification efficiency is low and the qPCR reaction conditions are not optimal. Redesign the primers and optimize the reaction conditions.

VIII. Limited Use License and Warranty

Limited Use License
Following terms and conditions apply to use of all OmicsLink™ ORF Expression Clones in all lentiviral vectors and Packaging Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty
GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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