



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-1200 (1200 qPCR reactions)

Performance optimized with All-In-One™ qPCR Primers and All-in-One First-Strand cDNA Synthesis Kit

User Manual I

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USER MANUAL I

All-in-One™ qPCR Mix

- I. Description
- II. Related Products
- III. Contents and Storage
- IV. Preparation
- V. Procedure
- VI. Example
- VII. Trouble Shooting Guide
- VIII. Limited Use License and Warranty

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. 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 human miRNAs. A careful process of co development ensures that they work well together and provide robust and reproducible results.

GeneCopoeia		Description
All-in-One™ First-Strand cDNA Synthesis Kit	Reverse transcription kit	Produces first-strand cDNA using poly A or total RNA as template
All-in-One™ qPCR Primers	Human, mouse and rat primers	Validated, gene-specific primers ensure specificity and sensitivity
All-in-One™ qPCR Primer Array	User specified, ready-to-use primer arrays in 96-well-plate format	Reliable tools ideal for analyzing the expression of a focused panel of genes such as pathways, diseases or customized gene panels
All-in-One™ miRNA qRT-PCR Detection Kits	SYBR® Green-based	Accurately quantify miRNA expression
All-in-One™ miRNA qPCR Primers	Human, mouse and rat primers	Validated for robust, reproducible and reliable quantitation of miRNA activity
OmicsLink™ Expression-Ready ORF cDNA Clones	20,000 human 15,000 mouse	Perform a variety of applications with expression-ready clones
Endofectin™ Transfection Reagents	Optimized for specific cell types	Transfect efficiently and with low toxicity

III. Contents and Storage

Contents and storage recommendations for the All-in-One qPCR Mix

Cat. Nos. AOPR-0200, AOPR-0600 and AOPR-1200 are provided in the following table.

Contents	Quantity	Storage temperature/ conditions
2X All-in-One qPCR Mix	2 x 1 ml 3 x (2 x 1 ml) 6 x (2 x 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.
50X ROX Reference Dye	1 x 80 µl 3 x 80 µl 6 x 80 ul	-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. Store the kit at -20°C. Avoid storage or leaving reagents at 4°C or room temperature. Avoid light exposure at all times.
2. Mix reagents thoroughly by gently inverting tubes several times avoiding bubbles and then briefly centrifuge before use.
3. Prepare the reaction mix with PCR grade water.
4. Strictly follow standard procedures for PCR to avoid nucleic acid contamination and non-specific amplification.
5. Read all procedures before setting up the PCR reaction

V. Procedure

1. Thaw the 2X All-in-One qPCR Mix and 50X ROX Reference Dye as needed.
2. Prepare the PCR reaction mix on ice. See the example below.

Reagent	Volume	Final concentration
2X All-in-One qPCR Mix	10 µl	1X
ddH ₂ O	1 µl	
PCR forward primer (2 µM)	2 µl	0.2 µM
PCR reverse primer (2 µM)	2 µl	0.2 µM
Template	5 µl	
50X ROX Reference Dye (if needed)	0.4 µl	1X
Total	20 µl	

- a. Use the 2X 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. ROX Reference Dye is used in real-time PCR instruments that require ROX for calibration.
 - c. 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).
 - d. 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.
 - e. 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 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.
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°C ~ 95°C	0.5°C/ time	6 sec/ time	Yes
25°C		30 sec	No

- ii. The DNA polymerase used in the 2X 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 80~150 bp. However, fragment lengths up to 300bp are possible.
- v. The main conditions 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 extension time and melting curve conditions accordingly.

VI. Example

Objective: The amplification efficiency and detection sensitivity of the 2X 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)

Procedure:

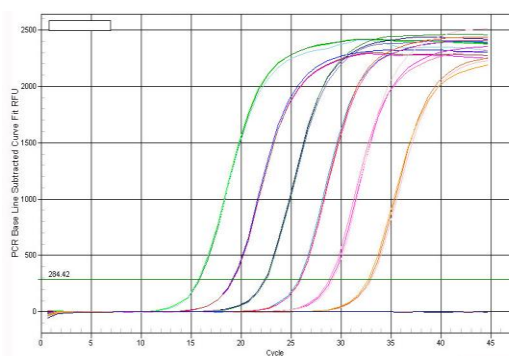
1. The plasmid is serially diluted to 6 concentrations ranging from 10⁵ to 1 molecule/μl.
2. PCR reaction mix preparation (on ice)

Reagent components	Volume
2X All-in-One qPCR Mix	10 µl
PCR forward primer (2 µM)	2 µl
PCR reverse primer (2 µM)	2 µl
ddH ₂ O	1 µl
Total	15 µl

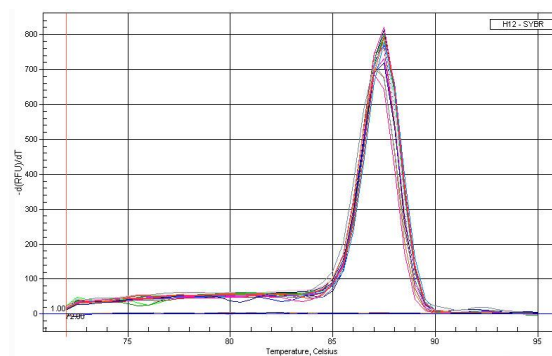
- Mix the above reagents sufficiently. Aliquot to PCR tubes after a brief centrifugation.
- Add 5 µl of the diluted plasmid template to each PCR tube. Use 5µl ddH₂O as a negative control.
- Program the PCR reaction and corresponding reading conditions of the melting curve:

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

- Analyze the amplification and corresponding melting curves after the qPCR experiment:

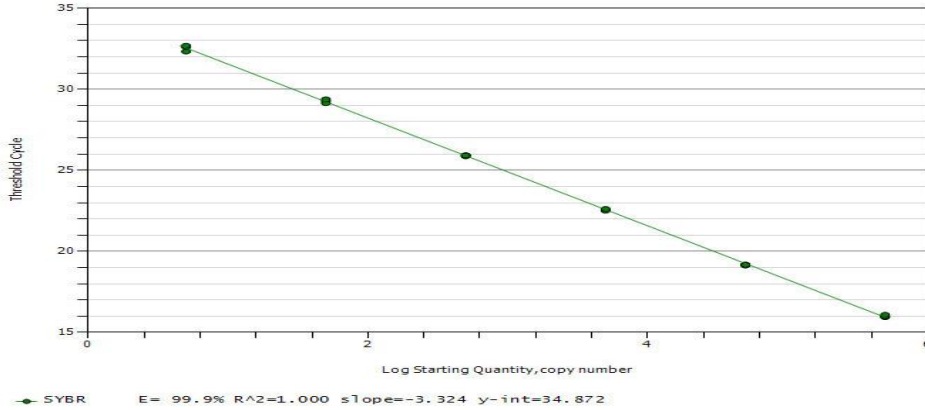


Amplification curves of serially diluted plasmid DNA



Peak values of amplified products in melting curves.

- 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

<p>Poor precision or failed qPCR reactions</p>	<ul style="list-style-type: none"> • 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.
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<p>Abnormal melting curves</p>	<p>Signal in the blank (No Template Control) sample</p> <ul style="list-style-type: none"> • 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 2X 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.
	<p>Double peaks and multiple peaks in the melting curve of the positive control</p> <ul style="list-style-type: none"> • 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.
<p>No signal (Ct) or late appearing signal</p>	<ul style="list-style-type: none"> • 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.

GeneCopoeia is committed to providing our customers with high-quality products. If you should have any questions or concerns about any GeneCopoeia products, please contact us at 301-762-0888.

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