All-in-One™ First-Strand cDNA Synthesis Kit for gene qPCR array

For reliable first-strand cDNA synthesis from all RNA sources

Cat.No.**QP008** (Old Cat.No.AORT-2020, 20 reverse transcription reations)
Cat.No.**QP009** (Old Cat.No.AORT-2060, 60 reverse transcription reations)

All-in-One™ qPCR Mix

For universal quantitative real-time PCR

Cat.No.**QP001** (Old Cat.No.AOPR-0200, 20 µl × 200 reactions)
Cat.No.**QP002** (Old Cat.No.AOPR-0600, 20 µl × 600 reactions)
Cat.No.**QP003** (Old Cat.No.AOPR-1200, 20 µl × 1200 reactions)
Cat.No.**QP004** (Old Cat.No.AOPR-1000, 20 µl × 1000 reactions)
Cat.No.**QP005** (Old Cat.No.AOPR-4000, 20 µl × 4000 reactions)

User Manual

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PART Ⅰ USER MANUAL

All-in-One™ First-Strand cDNA Synthesis Kit for gene qPCR array

I. Description

The All-in-One™ First-Strand cDNA Synthesis Kit includes a reverse transcriptase and a specialized set of reagents designed to yield first-strand cDNA that is optimal for gene cloning, cDNA library creation and quantitative PCR amplification. A robust experimental design delivers a universal kit that is suitable for first-strand cDNA synthesis from almost any source of RNA.

The kit uses Moloney Murine Leukemia Virus Reverse Transcriptase, RNase H Minus (M-MLV RTase (H–)) which is an RNA-dependent DNA polymerase used in cDNA synthesis with long RNA templates (>13kb). The lack of RNase H activity is important in this application in that RNase H activity will start to degrade template during long incubation times required for producing long cDNAs. RNase H minus RT enables preparation of long cDNAs and libraries containing a high percentage of full-length cDNA.

II. Related Products

Product	Description
All-in-One™ qPCR Mix	SYBR Green-based real-time quantitative PCR Mix
All-in-One™ qPCR Primers	Validated, gene-specific primers ensure specificity and sensitivity
RNAzol® RT RNA Isolation Reagent	Easy isolation of mRNA, microRNA and total RNA
ExProfile™ Gene qPCR Arrays	For expression profiling of pre-defined or customized sets of genes in various tissues or cells
All-in-One™ miRNA qRT-PCR Detection Kits	Accurately quantify miRNA expression
All-in-One™ miRNA qPCR Primers	Validated for robust, reproducible and reliable quantitation of miRNA activity
miProfile™ miRNA qPCR Arrays	For expression profiling of pre-defined or customized sets of miRNAs in various tissues or cells
OmicsLink™ Expression-Ready ORF cDNA Clones	Perform a variety of applications with expression-ready clones

III. Contents and Storage

Contents and storage recommendations for the All-in-One™ First-Strand cDNA Synthesis Kit (Cat. Nos. QP008 and QP009) are provided in the following table.

Contents	Quantity	Storage temperature/ conditions
200 U/µl M-MLV Reverse Transcriptase (RNase H-)	1 x 20 µl 3 x 20 µl	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at -80℃ in aliquots. Avoid repeated freezing/ thawing.
5X RT Reaction Buffer	1 x 100 µl 3 x 100 µl	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at -80℃ in aliquots. Avoid repeated freezing/ thawing.
25 U/µl RNase Inhibitor	1 x 20 µl 3 x 20 µl	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at -80℃ in aliquots. Avoid repeated freezing/ thawing.
25 mM dNTP	1 x 20 µl 3 x 20 µl	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at -80℃ in aliquots. Avoid repeated freezing/ thawing.
60 µM Oligo $(dT)_{18}$	1 x 20 µl 3 x 20 µl	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at -80℃ in aliquots. Avoid repeated freezing/ thawing.
250 µM Random Primer	1 x 20 µl 3 x 20 µl	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at -80℃ in aliquots. Avoid repeated freezing/ thawing.
Spike-in control RNA (for use with gene qPCR array only)	1 x 20 µl 3 x 20 µl	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at -80℃ in aliquots. Avoid repeated freezing/ thawing.
dd H_2O (RNase and DNase free)	1 x 1 ml 3 x 1 ml	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at -80℃ in aliquots. Avoid repeated freezing/ thawing.

IV. Preparation

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

RNA Sample Preparation

When working with RNA it is important to avoid RNases in your solutions, consumables and labware. When preparing your RNA samples, always wear a mask and disposable gloves in all procedures. Follow the described procedures you are using for RNA extraction carefully. Ready-to-use solutions that are RNase-free can be purchased. Alternatively treat solutions with diethyl pyrocarbonate (DEPC) and then autoclave. RNases on labware can also be inactivated by DEPC treatment or by baking at 250℃ for 3 hours. Use DEPC to treat all microcentrifuge tubes, pipettes and pipette tips (if not RNase free) and then autoclave to deactivate RNases. RNase-free consumables are available for purchase from many commercial sources.

IMPORTANT NOTES:

1. Store kit at –20℃. Avoid storage or leaving reagents at 4℃ or room temperature.
2. Mix reagents thoroughly by gently inverting tubes several times avoiding bubbles and then briefly centrifuge before use.
3. Set up all reactions on ice to reduce risk of RNA degradation.
4. Read all procedures before setting up RT reaction.

V. Procedure

1. Thaw all the reagents needed for RNA reverse transcription from the First-Stand cDNA Synthesis Kit. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Prepare the RNA-Primer Mix: Add the following reagents into an RNase-free reaction tube which has been pre-cooled on ice. The final volume should be 13μl.

Reagents	**Volume**	**Final concentration**
Total RNA or polyA RNA		1 μg or 10 ng †
250 μM Random Primer or 60 μM Oligo $(dT)_{18}$ or 10 μM sequence-specific primer	1 μl	10 μM or 2.4μM or 0.4 μM ††
Spike-in control RNA††† (Only comes in All-in-One first-strand cDNA synthesis kits for gene qPCR array)	1 μl	
ddH_2O (RNase/DNase free)	to 13 μl	

† The amount of RNA in the table is the recommended amount. The total RNA may be adjusted to between 10 ng ~ 5 µg, and the purified poly A RNA between 1 ng ~ 100 ng.

†† Please choose one of the RT Primers based on the experimental design. The reverse transcription will begin at the polyA tail if using the Oligo $(dT)_{18}$. It will begin at many different RT sites throughout the RNA if using the Random Primer.

††† Spike-in control RNA is used to monitor the efficiency of the RT reactions.

3. Denature RNA: Mix the reaction solution well. Spin down briefly. Heat the RNA-Primer mix at 65℃ for 10 minutes, then cool it down immediately on ice.

4. Prepare RNA reverse transcription reaction: Add the following reagents into the RNA-Primer mix reaction tube which has been cooled on ice. The final volume should be 25 µl.

Reagents	**Volume**	**Final concentration**
RNA-Primer Mix	13 µl	
5X RT Reaction Buffer	5 µl	1X
25 mM dNTP	1 µl	1 mM
25 U/µl RNase Inhibitor	1 µl	1 U
200 U/µl M-MLV RTase	1 µl	8 U
ddH_2O (RNase/DNase-free)	to 25 µl	

5. Reverse Transcription Reaction: Mix reaction solution well. Spin down briefly. Incubate the reaction solution at 37℃ for 60 minutes if using the Random Primer, or incubate at 42℃ for 60 minutes if using the oligo $(dT)_{18}$ or sequence-specific primer.

6. Terminate the reaction by heating at 85℃ for 5 minutes and then store at –20℃.

7. The cDNA reaction product can be used directly in the next step without being purified. A volume of 0.5 µl ~ 2 µl of undiluted cDNA is recommended for standard 25 µl PCR reactions. If performing quantitative PCR, it is recommended to do a 1:5 ~ 1:20 dilution of the cDNA and add a volume of 2 µl for each 20-µl qPCR reaction.

VI. Example

Objective: The reverse transcription efficiency of the All-in-One First Strand Synthesis Kit is assessed by examining the amplification results of different genes or gene regions using the oligo(dT) synthesized cDNA prepared from the All-in-One First-Strand cDNA Kit.

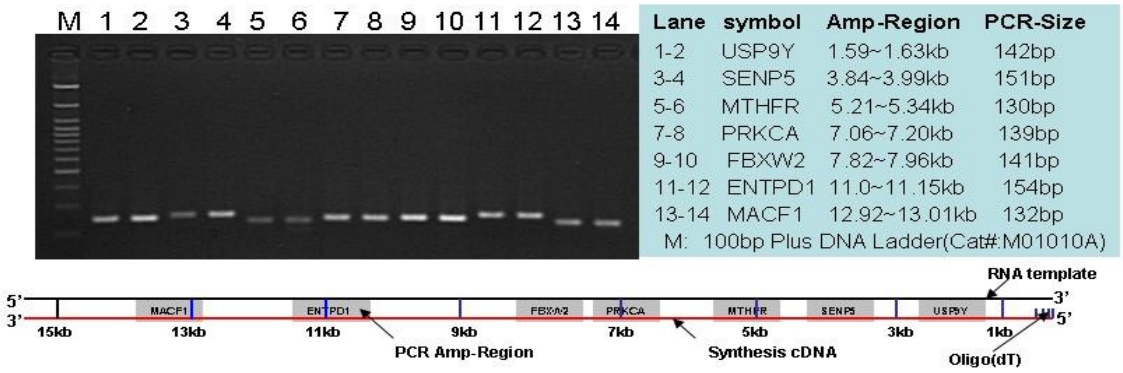

Figure 1. Efficient cDNA synthesis by All-in-One™ First-Strand cDNA Synthesis Kit. Total RNA isolated from human placenta was used as template RNA in reverse transcription reactions using the All-in-One First-Strand cDNA Synthesis Kit together with the oligo(dT) primer. The synthesized cDNA was then used to amplify different gene regions by quantitative PCR using the All-in-One qPCR Mix (GeneCopoeia Catalog No. QP001). The positive amplification results of MACF1 indicate that up to a 13 kb RNA sequence was reversed transcribed.

VII. Trouble Shooting Guide

Little or no RT-PCR product	**RNA template degradation** • The quality of the RNA is the key factor for cDNA synthesis. Follow the RNA isolation kit procedure carefully, always wearing a lab coat, gloves and mask when working with RNA and use RNA-Grade reagents and materials. Check the RNA quality by RNA electrophoresis in a denaturing gel. **An inhibitor was present in the RNA template** • Trace amounts of inhibitor such as guanidine salts in the RNA template can inhibit the cDNA synthesis. Re-precipitate the RNA with ethanol and wash the pellet with 75% ethanol. **A G-C rich template or secondary structure of the amplification product is obstructing the reaction** • Prepare the RNA-Primer Mix before the RT step. Then add a PCR enhancing reagent such as DMSO, betaine, etc. in the PCR reaction.
PCR product is longer than expected	• Genomic DNA was present. Perform a DNase I digest before the RT step or design intron-spanning or flanking primers to avoid co-amplification of genomic DNA. • The wrong product was amplified. Optimize the PCR reaction conditions.

VIII. Limited Use License and Warranty

Limited Use License
Following terms and conditions apply to use of all All-in-One™ First-Strand cDNA Synthesis Kits. 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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All-in-One™ qPCR Mix

For universal quantitative real-time PCR

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Cat.No.**QP002** (Old Cat.No.AOPR-0600, 20 µl × 600 reactions)
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User Manual I

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PART Ⅱ 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. 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 develop-ment 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™ 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
miProfile™ miRNA qPCR Arrays	User specified, ready-to-use primer arrays in 96 or 384 well plate	Reliable tools ideal for analyzing the expression of a focused panel of genes such as pathways, diseases or customized gene panels
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.QP001,QP002,QP003,QP004,QP005) are provided in the following table.

Contents	Quantity	Storage temperature/ conditions
2×All-in-One™ qPCR Mix	2×1 ml 3×(2×1 ml) 6×(2×1 ml) 5×(2×1 ml) 20×(2×1 ml)	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at –80℃ in aliquots. Avoid repeated freezing/ thawing.
50×ROX Reference Dye	1×80 μl 3×80 μl 6×80 μl 5×80 μl 20×80 μl	–20℃ (Stable for at least 12 months) Alternatively, the solution can also be stored at –80 ℃ 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℃. Avoid storage or leaving reagents at 4℃ 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 2×All-in-One qPCR™ Mix and 50×ROX Reference Dye as needed.**
2. **Prepare the PCR reaction mix on ice. See the example below.**

Reagent	Volume per reaction,	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	
50×Rox Reference Dye [e]	0.4 µl	1×
Water (double distilled)		
▪ Not using ROX Reference Dye	4 µl	
▪ Using ROX Reference Dye	3.6 µl	
Final volume	**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.

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 [a]	95℃	10 min	No
40	Denaturation	95℃	10 sec	No
	Annealing [b]	55℃~60℃	20 sec	No
	Extension	72℃	At least 15 sec [C]	Yes

a. The DNA polymerase used in the 2×All-in-One™ qPCR Mix is a special chemically modified hot-start enzyme. The indicted initial denaturation is sufficient to activate the enzyme.

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

c. The extension time is specific for the instrument. See the documentation provided with your instrument.

d. When using SYBR Green dye to monitor the qPCR reaction, a melting curve analysis should be performed immediately after qPCR cycling. For instructions, consult the documentation for your qPCR instrument. The following is an example adapted from the iQ5 real-time detection system from Bio-Rad Laboratories. The conditions for your instrument may differ.

Temperature range	Heating rate	Constant temperature	Detection
72–95℃	0.5℃/unit time	10 sec/unit time	Yes
25℃		30 sec	No

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**

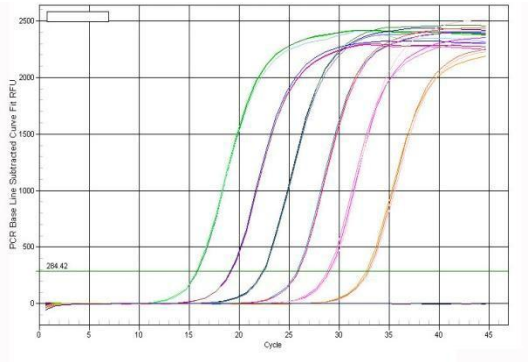
3. Mix the above reagents sufficiently. Aliquot to PCR tubes after a brief centrifugation.

4. Add 5 μl of the diluted plasmid template to each PCR tube. Use 5μl ddH_2O as a negative control.

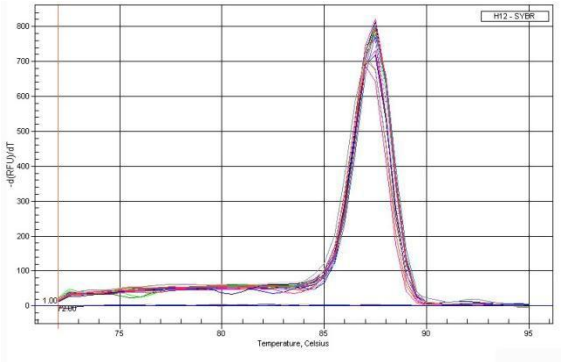
5. Program the PCR reaction and corresponding reading conditions of the melting curve:

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

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

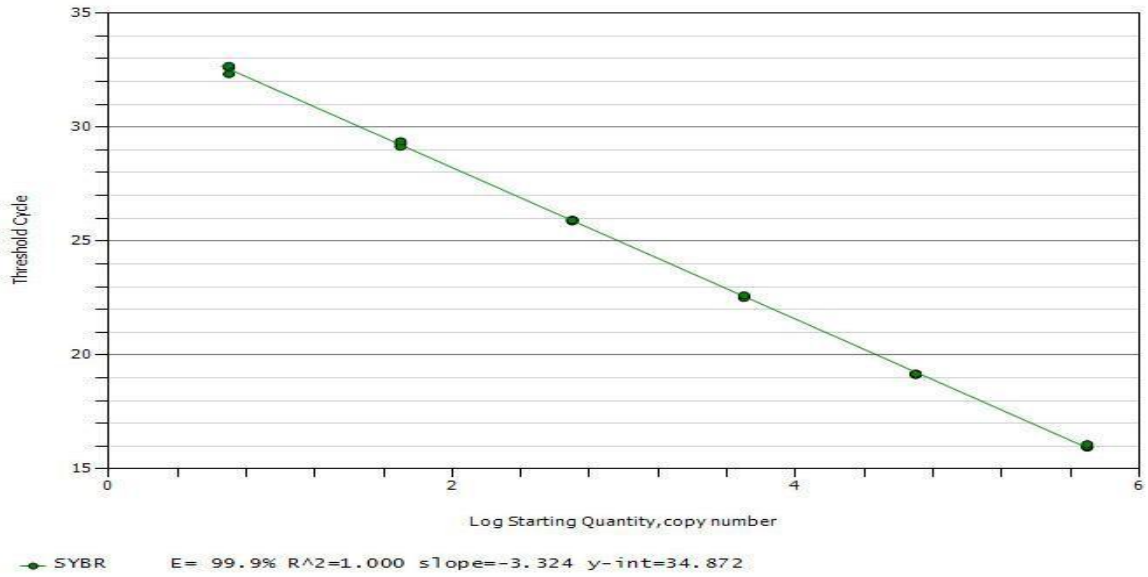


Amplification curves of serially diluted plasmid DNA



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	• 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 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
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