BlazeTaq™ One-Step SYBR® Green RT-qPCR kit

Easy-to-use mixes for dye-based real-time PCR and RT-qPCR

With ROX Reference Dye	Without ROX Reference Dye
Cat.No.**QP071** (20 µl × 200 reactions)	Cat.No.**QP081** (20 µl × 200 reactions)
Cat.No.**QP072** (20 µl × 600 reactions)	Cat.No.**QP082** (20 µl × 600 reactions)
Cat.No.**QP074** (20 µl × 1000 reactions)	Cat.No.**QP084** (20 µl × 1000 reactions)

Performance optimized for All-In-One™ qPCR Primers, ExProfile™ Gene qPCR Arrays.

User Manual

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USER MANUAL

BlazeTaq™ One-Step SYBR® Green RT-qPCR kit

I. Description

BlazeTaq™ One-Step SYBR® Green RT-qPCR kit is based on quantitative reverse transcription PCR (RT-qPCR) that uses RNA as starting material. It offers a convenient master mix to convert RNA to DNA and quantify in a one step reaction. The kit is supplied with reverse transcriptase and a 5X master mix with Hot-Start Taq DNA polymerase, dNTP and all required buffer components, and it is universally compatible to all instrument platforms. This kit uses a reverse transcriptase to convert RNA to DNA, and an antibody-modified Taq DNA polymerase to avoid polymerase activity prior to thermal cycling. Upon heating to 95 ºC for 3 min, the antibody dissociates and full activity of the Taq polymerase is restored. Also, the optimized buffer system allows high amplification efficiency and specificity, as well as enhanced sensitivity of real time PCR reactions over a wide range of templates.

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
BlazeTaq™ SYBR® Green qPCR mix	SYBR Green-based real-time quantitative PCR Mix
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

1. Contents and storage recommendations for the BlazeTaq™ One-Step SYBR® Green RT-qPCR kit are provided in the following table.

For kits with the catalog number **QP071**, **QP072** and **QP074**

Catalog Number	Contents	Quantity	Storage temperature/ conditions
QP071-01	BlazeTaq One Step RT-qPCR Mix (5×)	1×800 μL 3×(1×800 μL) 5×(1×800 μL)	Store at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP071-02	BlazeTaq RTase Mix (50×)	1×80 μL 3×(1×80 μL) 5×(1×80 μL)	Store at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP001-02	ROX Reference Dye (30 μM)	1×80 μL 3×(1×80 μL) 5×(1×80 μL)	Store in dark at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.

2. Contents and storage recommendations for the BlazeTaq™ One-Step SYBR® Green RT-qPCR kit (without ROX) are provided in the following table.

For kits with the catalog number **QP081**, **QP082** and **QP084**

Catalog Number	Contents	Quantity	Storage temperature/ conditions
QP071-01	BlazeTaq One Step RT-qPCR Mix (5×)	1×800 μL 3×(1×800 μL) 5×(1×800 μL)	Store at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.
QP071-02	BlazeTaq RTase Mix (50×)	1×80 μL 3×(1×80 μL) 5×(1×80 μL)	Store at -20°C (Stable for at least 12 months). Alternatively, the solution can also be stored at -80°C in aliquots. Avoid repeated freezing/thawing.

Required Materials (Not Included)

- RNA template
- Target-specifc forward and reverse primers
- ddH_2O (Nuclease-free)
- PCR strip tubes or microcentrifuge tubes (for reaction setup)
- qPCR tubes or plates (Rnase-free)
- nuclease-free pipettors
- Microcentrifuge
- qPCR instrument

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°C 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 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 while avoiding bubbles, and then briefly centrifuge before use.

3. Prepare the reaction mix with PCR grade water.

4. RT-qPCR is a sensitive RNA detection method. Set up all reactions on ice to reduce risk of RNA degradation.

5. A denaturation or melt curve step should be added at the end of the RT-qPCR cycling protocol to evaluate amplifcation specifcity.

6. Read all procedures before setting up the PCR reaction.

V. Procedure

1. Thaw the BlazeTaq One Step RT-qPCR Mix (5×) and other reaction components at room temperature, then place on ice. After thawing completely, briefly mix each component by inversion, pipetting or gentle vortexing.

2. Prepare the RT-qPCR reaction mix according to the table below. Add the following reagents into an RNase-free reaction tube or plate which has been pre-cooled on ice.

Reagent	Volume [a]	Final concentration
BlazeTaq One Step RT-qPCR Mix (5×)	4 µl	1×
BlazeTaq RTase Mix (50×)	0.4 ul	1×
PCR forward primer (10 µM) [b]	0.4 µl	0.2 µM [c]
PCR reverse primer (10 µM)	0.4 µl	0.2 µM

RNA Template [d]	5 µl	variable
ROX Reference Dye [e](30 µM), *optional*	0.4 - 0.1 µl	600 nM - 150 nM
dd H_2O		
▪ Not using ROX Reference Dye	9.8 µl	
▪ Using ROX Reference Dye	9.4-9.7 µl	
Total	**20 µl**	

a. The kit has been optimized for a final reaction volume of 20 µl. If the total reaction volume is changed, maintain each component in the proper proportion.

b. Primers are important considerations to ensure success with one step RT-qPCR. 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. The detection range of this kit can be from 1 pg to 100 ng, depending on target abundance and RNA quality. Generally, the recommended amount of RNA template can be ranged from 1 ng to 10 ng. RNA template can be quantitated using UV absorbance at 260 nm. RNA quality can be analyzed using a bioanalyzer or agarose gel electrophoresis.

e. ROX Reference Dye is only supplied in BlazeTaq™ One-Step SYBR® Green RT-qPCR kit (Cat. Nos.QP071, QP072 and QP074). It should be 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 20 µl 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 (0.2-0.4 µl)	600 nM (300-600 nM)
ABI 7500, 7500 Fast, ABI ViiA7, Stratagene Mx3000P, Mx3005P, Mx4000	0.1 µl (0.02-0.1 µl)	150 nM (30-150 nM)

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

f. Prepare control reactions as follows if needed:

No-RT controls: To test for genomic DNA contamination of the RNA sample, do not add the BlazeTaq RTase Mix (50×).

No-template controls: To test for genomic DNA contamination of the reaction mixes, do not add RNA template.

3. Mix the RT-qPCR reaction mix sufficiently and add to the PCR reaction tubes.

4. Briefly centrifuge to remove bubbles and make sure all the reagents are at the bottom of the reaction tubes/plates.

5. The following method for programming the RT-qPCR reaction is recommended:

Cycles	Steps	Temperature	Time	Detection
1	Reverse Transcription	42°C	10 min	No
1	Initial Denaturation	95°C	3 min	No
40	Denaturation	95°C	10 sec	No
	Extension	60°C	30 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, please consult the documentation of your qPCR instrument for instructions.

ii. A 42°C RT step temperature is optimal for reverse transcriptase. To insure best performance and full activation, avoid using a temperature of < 42°C.

iii. The optimal fragment length to use for amplification during RT-qPCR is in the range of 80-150 bp. However, fragment lengths up to 500 bp are possible.

iv. The main condition for the above reaction are referred to 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 BlazeTaq One-Step SYBR Green RT-qPCR kit are assessed by examining the amplification result of GAPDH from serially diluted total RNA sample extracted from HeLa cells.

Equipment: iQ5 instrument (Bio-Rad Laboratories)

Procedure:

1. The amount of total RNA used for GAPDH detection was ranged from 10 ng to 0.1 pg (10-fold serially diluted to 6 concentrations).

2. RT-qPCR reaction mix was prepared as below.

Reagent components	**Volume**
BlazeTaq One Step RT-qPCR Mix (5×)	4 µl
BlazeTaq RTase Mix (50×)	0.4 ul
PCR forward primer (10 µM)	0.4 µl
PCR reverse primer (10 µM)	0.4 µl
ddH_2O	9.8 µl
Total	**15 µl**

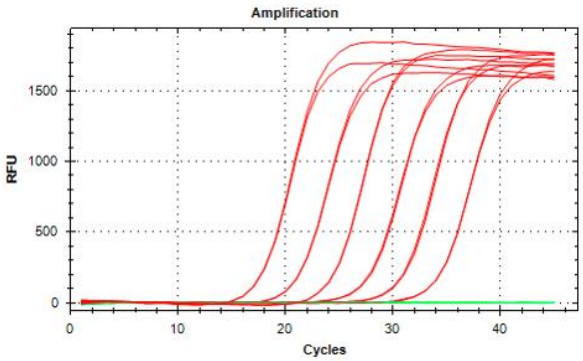
3. Mix the above reagents sufficiently. Aliquot to PCR tubes after a brief centrifugation.

4. Add 5 µl of the total RNA to each PCR tube. Use 5 µl ddH_2O as a no-template control (NTC).

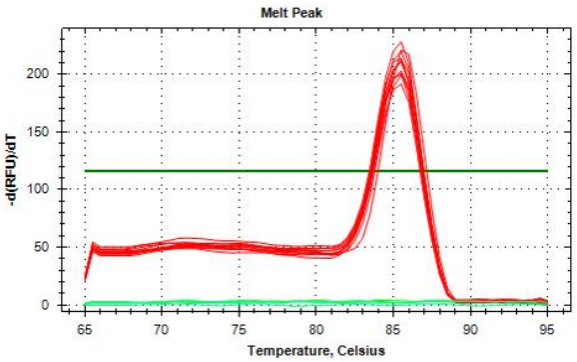
5. Program the RT-qPCR reaction and corresponding reading conditions of the melting curve:

Cycles	**Steps**	**Temperature**	**Time**	**Detection**
1	Reverse Transcription	42°C	10 min	No
1	Initial Denaturation	95°C	3 min	No
45	Denaturation	95°C	10 sec	No
	Extension	60°C	30 sec	Yes
1	Melting Curve Analysis	72°C~95°C	**Heating Rate** 0.5°C /unit time	Yes
	Cooling	25°C	30 sec	No

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

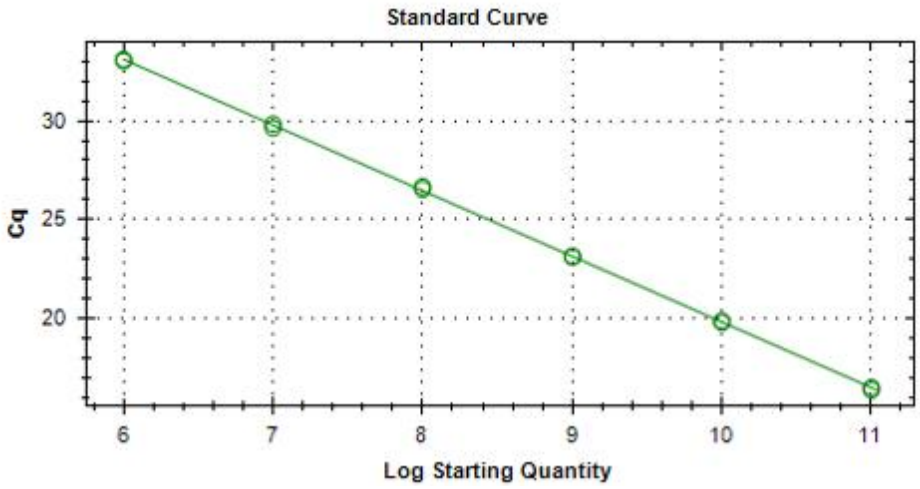


Amplification curves of serially diluted Total RNA.



Peak values of amplified products in melting curves.

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



8. **Conclusion**: The peak values from the amplification and melting curves show that BlazeTaq One-Step SYBR Green RT-qPCR kit can detect GAPDH from as low as 1 pg of total RNA, showing that very high sensitivity can be attained using the BlazeTaq One-Step SYBR Green RT-qPCR kit. At the same time, high amplification efficiency is also shown by the good linear relationship among each concentration of serially diluted total RNA.

VII. Trouble Shooting Guide

Problem	Possible Cause	Solution
No or low RT-qPCR product (signal)	RNA template has been damaged/degraded.	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.
	There is not enough RNA template.	After increasing the number of cycles has shown no success, repeat the reaction with a higher concentration of template.
	RNase contamination	Maintain aseptic conditions; add RNase inhibitor.
	Primer design is not optimal.	Confirm the accuracy of the sequence information and the specificity of the primer sequence to non-target sequences.
	RT-qPCR product is too long.	The best results are obtained when RT-qPCR products are between 80-150 bp, and do not exceed 500 bp.
	A G-C rich template or secondary structure of the amplification product is obstructing the reaction.	Prepare the RNA-Primer Mix before the RT-qPCR reaction. Then add a qPCR enhancing reagent such as DMSO, betaine, etc. in the qPCR reaction.
RT-qPCR 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.
Product detected at lower-than expected cycle number, and/or positive signal from no template controls	RNA template or RT-qPCR reaction carry-over contamination.	Maintain aseptic conditions. Use separate dedicated pipettors for RT-qPCR reactions. Assemble reactions (except for target addition) in a DNA-free area.

VIII. Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of all BlazeTaq™ One-Step SYBR® Green RT-qPCR kits (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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