CytoCt™ RT-qPCR System User Manual

To analyze gene expression by real-time RT-qPCR directly from cultured cells

CytoCt™ Cell Lysis Kit

Cat. No: QP309 (20 reactions), QP310 (100 reactions)

CytoCt™ cDNA Synthesis Kit

Cat. No: QP356 (20 reactions)

CytoCt™ Probe One-Step RT-qPCR Kit

Cat. No: QP375 (100 reactions)

CytoCt™ One-Step SYBR® Green RT-qPCR Kit

Cat. No: QP370 (100 reactions)

User Manual

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CytoCt™ RT-qPCR System

I. Description

GeneCopoeia's CytoCt™ RT-qPCR System allows you to analyze gene expression by real-time RT-qPCR directly from 10-100,000 cultured cells either in tubes or 96-well plates without any RNA purification, providing enhanced convenience, speed, throughput, data reliability, and sensitivity. The system includes the CytoCt™ Cell Lysis Kit, CytoCt™ cDNA Synthesis Kit, CytoCt™ Probe One-Step RT-qPCR Kit, and the CytoCt™ One-Step SYBR® Green RT-qPCR Kit.

II. Contents and Storage

Store all components at -20°C (stable for at least 12 months). Avoid repeated freezing/ thawing.

Cat. No.	Product	Content No.	Contents	Part No.	Quantity
QP309	**CytoCt™ Cell Lysis Kit (20 rxns)**	N/A	CytoCt lysis buffer	QP309-01	1x 1mL
		N/A	DNase I	QP309-02	1x 40 µl
QP310	**CytoCt™ Cell Lysis Kit (100 rxns)**	N/A	CytoCt lysis buffer	QP309-01	5x 1 ml
		N/A	DNase I	QP310-02	1x 200 uL
QP356	**CytoCt™ cDNA Synthesis Kit (20 rxns)**	QP309	CytoCt lysis buffer	QP309-01	1x 1 ml
			DNase I	QP309-02	1x 40 µl
		QP056	SureScript RTase mix (20×)	QP056-01	1x 20 µl
			SureScript RT reaction buffer (5×)	QP056-02	1x 80 µl
			ddH2O (RNase/DNase free)	QP006-07	1x 1 ml
QP375	**CytoCt™ Probe One-Step RT-qPCR Kit (100 rxns)**	QP310	CytoCt lysis buffer	QP309-01	5x 1 ml
			DNase I	QP310-02	1x 200 µl
		QP075	Probe One-Step RT-qPCR mix (5×)	QP075-01	1x 400 µl
			BlazeTaq One-Step RTase mix	QP075-02	1x 40 µl
			ROX reference dye	QP070-03	1x 40 µl

QP370	CytoCt™ One-Step SYBR® Green RT-qPCR Kit (100 rxns)	QP310	CytoCt lysis buffer	QP309-01	5x 1 ml
			DNase I	QP310-02	1x 200 µl
		QP070	BlazeTaq One-Step RT-qPCR mix	QP070-01	1x 400 µl
			BlazeTaq RTase mix (50×)	QP070-02	1x 40 µl
			ROX reference dye	QP070-03	1x 40 µl

III. Preparation

1) Sterile disposable plastic containers without RNase should be used as often as possible. If RNase cannot be removed, sterilization should be carried out before use. If glassware is used, it should be sterilized by dry heat at 160 ℃ for at least 2 hours, or treated with 0.1% DEPC treated water at 37 ℃ for 12 hours, then autoclaved.
2) It is recommended that any apparatus and instruments be used strictly for RNA experiments and not for any other purpose.
3) Use 0.1% DEPC treated water to prepare reagents, followed by autoclaving before use. If the reagent cannot be autoclaved, prepare in a sterile container with sterile water, followed by filtration before use.
4) Wearing a lab coat, disposable gloves and protective goggles are recommended when handling chemicals.

Sample Preparation

Harvest cells in PBS and count the cell numbers.

IMPORTANT NOTES:

1) Store the kit at -20°C. Avoid storing or leaving reagents at 4°C or room temperature.
2) Mix reagents thoroughly by gently inverting tubes several times while avoiding bubbles. Briefly centrifuge before use.
3) Set up all reactions on ice to reduce risk of RNA degradation.
4) Read all procedures before setting up the RT reaction.

IV. Procedure

1. Make CytoCt **Lysis Buffer Mix**:
   For each reaction, mix 50 µl of **Cell Lysis Buffer** with 2µl of **DNase I**. Place on ice. Use within 2 hours.

   a. **Processing of nonadherent cells in tubes**

   - Count the cells. Transfer 10–100,000 cells per well (tube) to a 96-well PCR plate or PCR tubes.
   - Centrifuge at 1,000 x g for 5-10 min. Remove as much of the medium as possible without disturbing the cell pellet.
   - Wash cells with 125 µl of room temperature PBS. Centrifuge at 1,000 x g for 5-10 min. Carefully remove the PBS buffer without disturbing the cell pellet.
   - Add 50 µl of CytoCt Lysis Buffer Mix to each well or tube.
   - Pipet up and down five times to ensure complete resuspension of the cell pellet.
   - Incubate at room temperature for 10 min.
   - Go to Step 2

   b. Processing of adherent cells in a 96-well culture plate:

   - Seed the cell culture in advance in a 96-well culture plate such that the cells divide to reach 50% - 90% confluency.
   - Remove cell culture medium completely by aspiration.
   - Wash cells with 125 µl of room temperature PBS twice. Aspirate to remove PBS completely.
   - Add 50 µl of CytoCt **Lysis Buffer Mix** (with DNase I) to each of the wells. Rock the plates several times to let CytoCt Lysis Buffer Mix cover the cells.
   - Incubate at room temperature for 10 min,
   - Transfer each cell lysate to a PCR plate,
   - Go to Step 2

2. Incubate in a thermocycler following the procedure below.

Incubation at	Time
25°C	2 min
37°C	5 min
75°C	10 min

Note: The lysate can be stored on ice up to 6h, or -20°C up to 48h. Store at -80°C for up to one month.

3. The lysate can be directly used for RT reactions or one-step RT-qPCR reactions.
For RT reactions:

(1) Use 1-5 µl of cell lysate in each RT reaction. Prepare the RT reaction mix according to the table below.

SureScript™ First-Strand cDNA Synthesis Kit	Volume	Final concentration
SureScript RTase Mix (20×)	1 µl	1×
SureScript RT Reaction Buffer (5×)	4 µl	1×
Cell Lysate	**1-5 µl**	-
ddH2O (RNase/DNase free)	to **20 µl**	-

Notes: The sensitivity of this kit ranges from 10 cells to 100,000 cells, depending on target abundance and RNA quality. Generally, the amount of the cell lysate in the table is the recommended amount to use. The total volume of lysate may be adjusted to between 1-5 µl.

(2) The following programming parameters for the reverse transcription reaction are recommended:

Temperature	Time
25℃	5 min
42℃	15 min*
85℃	5 min
4℃	hold

*Generally, 15 minutes is sufficient for the reverse transcription reaction for most samples. For more complex or longer templates, the reaction time can be extended appropriately according to the needs of the specific situation, generally not more than 60 minutes.

(3) The RT reaction product can be used directly in the next step without being purified. A volume of 0.5 µl ~ 2 µl of the 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.

For one-step RT-qPCR:

Dilute the lysates 5 fold with ddH2O, then use 1-2 µl in each one-step RT-qPCR reaction.

(1) Using the **BlazeTaq™ One-Step SYBR® Green RT-qPCR Kit** or **BlazeTaq™ Probe One-Step RT-qPCR Kit** as examples, prepare the RT-qPCR reaction mix according to the table below.

Reagent	Volume [a]	Final concentration
BlazeTaq One Step RT-qPCR Mix (5×)	4 µl	1x
BlazeTaq RTase Mix (50×)	0.4 µl	1x
PCR forward primer (10 µM) [b]	0.4 µl	0.2 µM [c]
PCR reverse primer (10 µM)	0.4 µl	0.2 µM
Cell lysate	1-2 µl	variable
ROX reference dye [d](30 µM), *optional*	0.4 - 0.1 µl	
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 transcripts. When 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. ROX reference dye is only supplied in BlazeTaq™ One-Step SYBR® Green RT-qPCR kit (Cat. Nos. QP070, 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 with ROX but are not listed in the table, please optimize the concentration of ROX according to the guidelines of the specific instrument.

e. Prepare control reactions as follows if needed:

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

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

(2) Mix the RT-qPCR reaction mix sufficiently and add to the PCR reaction tubes.

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

(4) 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:

a. 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.

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

c. 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.

d. The primary 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. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to use of CytoCt™ RT-qPCR System (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.

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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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