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: QP209 (20 reactions), QP210 (100 reactions)

CytoCt™ cDNA Synthesis Kit

Cat. No: QP256 (20 reactions)

CytoCt™ Probe One-Step RT-qPCR Kit

Cat. No: QP275 (100 reactions), QP285 (without ROX, 100 reactions)

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

Cat. No: QP270 (100 reactions), QP280 (without ROX, 100 reactions)

User Manual

GeneCopoeia, Inc.
9620 Medical Center Drive, #101 Rockville, MD 20850
USA

301-762-0888

inquiry@genecopoeia.com

www.genecopoeia.com

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 10-10,000 of cultured cells without any RNA purification, providing enhanced convenience, speed, 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
QP209	**CytoCt™ Cell Lysis Kit (20 rxns)**	N/A	CytoCt lysis buffer	QP256-01	1x 200 µl
		N/A	DNase I	QP256-02	1x 10 µl
QP210	**CytoCt™ Cell Lysis Kit (100 rxns)**	N/A	CytoCt lysis buffer	QP275-01	1x 1 ml
		N/A	DNase I	QP275-02	1x 50 µl
QP256	**CytoCt™ cDNA Synthesis Kit (20 rxns)**	QP209	CytoCt lysis buffer	QP256-01	1x 200 µl
			DNase I	QP256-02	1x 10 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
QP275	**CytoCt™ Probe One-Step RT-qPCR Kit (100 rxns)**	QP210	CytoCt lysis buffer	QP275-01	1x 1 ml
			DNase I	QP275-02	1x 50 µl
		QP075	Probe One-Step RT-qPCR mix (5×)	QP075-01	1x 400 µl
			BlazeTaq One-Step RTase mix (50×)	QP075-02	1x 40 µl
			ROX reference dye	QP070-03	1x 40 µl
QP285	**CytoCt™ Probe One-Step RT-qPCR Kit (without ROX, 100 rxns)**	QP210	CytoCt lysis buffer	QP275-01	1x 1 ml
			DNase I	QP275-02	1x 50 µl
		QP085	Probe One-Step RT-qPCR mix (5×)	QP075-01	1x 400 µl
			BlazeTaq One-Step RTase mix (50×)	QP075-02	1x 40 µl
QP270	**CytoCt™ One-Step SYBR® Green RT-qPCR Kit (100 rxns)**	QP210	CytoCt lysis buffer	QP275-01	1x 1 ml
			DNase I	QP275-02	1x 50 µl
		QP070	BlazeTaq One-Step RT-qPCR mix (5×)	QP070-01	1x 400 µl
			BlazeTaq RTase mix (50×)	QP070-02	1x 40 µl
			ROX reference dye	QP070-03	1x 40 µl
QP280	**CytoCt™ One-Step SYBR® Green RT-qPCR Kit (without ROX, 100 rxns)**	QP210	CytoCt lysis buffer	QP275-01	1x 1 ml
			DNase I	QP275-02	1x 50 µl
		QP080	BlazeTaq One-Step RT-qPCR mix (5×)	QP070-01	1x 400 µl
			BlazeTaq RTase mix (50×)	QP070-02	40 µl

III. Preparation

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

Sample Preparation

When preparing your samples, always wear a lab coat and disposable gloves in all procedures. Harvest cells in PBS buffer 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 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 the RT reaction.

IV. Procedure

1. Make **Lysis Buffer Mix**: for each reaction, mix 10 µl of **Cell Lysis Buffer** with 0.5 µl of **DNase I**.
2. Suspend cells in PBS at concentration of 10 – 10,000 cells/µl (typically 2,000 – 5,000 cells/µl), and mix well to ensure complete suspension of the cell pellet.
3. Add 10 µl of **Lysis Buffer Mix** (with DNAse I) to 2 µl cells on ice and mix well, then incubate according to the following procedure.

Incubation at	**time**
25°C	5 min
37°C	5 min
75°C	10 min

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

4. The lysate can be directly used for RT reactions or One-step RT-qPCR:

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 10,000 cells, depending on target abundance and RNA quality. Generally, the amount of the cell lysate in the table is the recommended amount. The total lysate may be adjusted to between 1-5 µl lysate.

(2) The following method for programming the reverse transcription reaction is recommended:

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

*Generally, 15 minutes is sufficient for the reverse transcription reaction of most samples. For more complex or longer templates, the reaction time can be prolonged appropriately according to the actual 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 times with ddH2O, then use 1-2 µl in each One-step RT-qPCR.

(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	1×
BlazeTaq RTase Mix (50×)	0.4 µl	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
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 genes. 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 of ROX but have not been listed out 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 (50×).

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 main condition for the above reaction is 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 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.

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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GeneCopoeia, Inc.
9620 Medical Center Drive, #101, Rockville, MD 20850
Tel: 301-762-0888 Fax: 301-762-3888,
Email: inquiry@genecopoeia.com
Web: www.genecopoeia.com