# ExoCt™ RT-qPCR System User Manual

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

## ExoCt™ First-Strand cDNA Synthesis Kit

Cat. No: QP125 (20 reactions)

Cat. No: QP126 (60 reactions)

## ExoCt™ SYBR® Green RT-qPCR Kit

Cat. No: QP131 (20 RT and 200 qPCR reactions)

Cat. No: QP132 (60 RT and 600 qPCR reactions)

**User Manual**

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# ExoCt™ RT-qPCR System



## I. Description

GeneCopoeia's ExoCt™ RT-qPCR System allows you to analyze gene expression by real-time qPCR directly from exosomes without any RNA purification. The ExoCt™ First-Strand cDNA Synthesis Kit contains ExoCt™ RT-for-All™ Buffer and ExoCt™ PAP/RTase Mix, which were specifically developed for robust first-strand cDNA synthesis covering a variety of RNA templates, including miRNA, mRNA, LncRNA, etc., from exosome lysates in a single reaction. The ExoCt™ SYBR® Green RT-qPCR Kit includes both RT and qPCR reagents, which combines PCR technology and SYBR® Green for fast and accurate quantification of exosome RNAs. The system includes the ExoCt™ First-Strand cDNA Synthesis Kit and the ExoCt™ 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 |
|---|---|---|---|---|---|
| QP125 | ExoCt™ First-Strand cDNA Synthesis Kit (20 reactions) | N/A | ExoCt™ Lysis Buffer | QP125-01 | 1×100 µl |
| | | N/A | DNase I | QP125-02 | 1×10 µl |
| | | N/A | 5× ExoCt™ RT-for-All™ Buffer | QP125-03 | 1×80 µl |
| | | N/A | 20× ExoCt™ PAP/RTase Mix | QP125-04 | 1×20 µl |
| | | N/A | ddH2O (RNase/DNase free) | QP006-07 | 1×1 ml |
| QP126 | ExoCt™ First-Strand cDNA Synthesis Kit (60 reactions) | N/A | ExoCt™ Lysis Buffer | QP125-01 | 3×100 µl |
| | | N/A | DNase I | QP125-02 | 3×10 µl |
| | | N/A | 5× ExoCt™ RT-for-All™ Buffer | QP125-03 | 3×80 µl |
| | | N/A | 20× ExoCt™ PAP/RTase Mix | QP125-04 | 3×20 µl |
| | | N/A | ddH2O (RNase/DNase free) | QP006-07 | 3×1 ml |
| QP131 | ExoCt™ SYBR® Green RT-qPCR Kit (20 RT and 200 qPCR reactions) | QP125 | ExoCt™ Lysis Buffer | QP125-01 | 1×100 µl |
| | | | DNase I | QP125-02 | 1×10 µl |
| | | | 5× ExoCt™ RT-for-All™ Buffer | QP125-03 | 1×80 µl |
| | | | 20× ExoCt™ PAP/RTase Mix | QP125-04 | 1×20 µl |
| | | | ddH2O (RNase/DNase free) | QP006-07 | 1×1 ml |
| | | QP031 | 5× BlazeTaq™ qPCR Mix | QP031-01 | 1×800 µl |
| | | | ROX Reference Dye | QP001-02 | 1×80 µl |
| | | QP029 | Universal Adaptor PCR Primer | QP029 | 1×20 µl |

| | | | | | |
|---|---|---|---|---|---|
| QP132 | ExoCt™ SYBR® Green RT-qPCR Kit (60 RT and 600 qPCR reactions) | QP126 | ExoCt™ Lysis Buffer | QP125-01 | 3×100 µl |
| | | | DNase I | QP125-02 | 3×10 µl |
| | | | 5× ExoCt™ RT-for-All™ Buffer | QP125-03 | 3×80 µl |
| | | | 20× ExoCt™ PAP/RTase Mix | QP125-04 | 3×20 µl |
| | | | ddH2O (RNase/DNase free) | QP006-07 | 3×1 ml |
| | | QP032 | 5× BlazeTaq™ qPCR Mix | QP031-01 | 3×800 µl |
| | | | ROX Reference Dye | QP001-02 | 3×80 µl |
| | | QP029 | Universal Adaptor PCR Primer | QP029 | 3×20 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°C for at least 2 hours, or treated with 0.1% DEPC treated water at 37°C for 12 hours, and then autoclaved.
2. GeneCopoeia recommends that the apparatus and instruments used in this procedure should be dedicated for RNA work and not for any other experiments.
3. Where possible, reagents should be prepared using 0.1% DEPC treated water, followed by autoclaving before use. If any reagents cannot be autoclaved, they should be sterilized by filtration.
4. Wearing a lab coat, disposable gloves and protective goggles are recommended when handling chemicals.

**Sample Preparation**

1. Purify exosomes from cell culture medium or other fluids.
   **Note:** GeneCopoeia recommends using the ExoSure™ Exosome Isolation Kit (Cat. No. EP002) for this purpose.
2. Exosomes purified using other methods should be resuspended in 1× PBS.
   Note: Exosomes purified from 0.5-1 ml of medium should be resuspended in 5-10 µl of PBS buffer. Use 5 µl in the ExoCt™ Exosome Lysis reactions.

**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. Procedures

### Exosome Lysis reactions

1. Transfer 5 µl of exosomes to a new PCR tube, and add 5ul of ExoCt™ Lysis Buffer.
2. Add 0.5 µl of DNase I
   Note: for miRNA detection, DNase I is optional
3. Mix well and briefly spin.
4. In a thermal cycler, incubate at 37°C for 10 min, 75°C for 10 min, then cool to 4°C.
5. Place tubes on ice.
6. Use 1-8 µl of lysate for the RT-for-All™ reaction.

### RT-For-All™ reverse transcription reactions

1. For each reaction, set up the reagents below in a PCR plate:

| Reagent | Volume | Final concentration |
|---|---|---|
| 5× ExoCt™ RT-for-All™ Buffer | 4 µl | 1× |
| 20× ExoCt™ PAP/RTase Mix | 1 µl | 1× |
| Exosome lysates | 5 µl (1-8 µl) | - |
| dd$H_2O$ (RNase/DNase free) | to 20 µl | - |

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

| Temperature | Time |
|---|---|
| 37°C | 30 min |
| 85°C | 5 min |
| 4°C | hold |

**Note:** The RT-For-All™ reactions can be stored at -80°C for more than 6 months after this step.

3. Dilute 2-5 times. Use 2 µl for qPCR.
   **Note:** The RT-For-All™ reactions can efficiently reverse transcribe most RNA species in exosomes, including miRNA, mRNA, LncRNA, etc.

### qPCR reactions

1. Thaw the 5× BlazeTaq™ qPCR Mix and ROX Reference Dye as needed.
2. Prepare the PCR reaction mix on ice. Refer to the table below.

| Reagent | Volume | Final concentration |
|---|---|---|
| 5× BlazeTaq™ qPCR Mix[a] | 4 µl | 1× |
| PCR forward primer (2 µM)[b] | 2 µl | 0.2 µM |
| PCR reverse primer (2 µM)[c] | 2 µl | 0.2 µM[d] |
| Template[e] | | |
| ROX Reference Dye[f] (30 µM), optional | 0.4 - 0.1 µl | 600 -150 nM |
| Water (double distilled) | | |
| ■ Not Using ROX Reference Dye | 10 µl | |
| ■ Using ROX Reference Dye | 9.6 - 9.9 µl | |
| Total | 20 µl | |

a. Use the 5× BlazeTaq qPCR Mix as one-fifth 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 critically important for ensurings 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. If you are designing your own primers, GeneCopoeia recommends using Oligo primer analysis software (Molecular Biology Insights) or Primer Premier software (Premier Biosoft International).

c. For amplifying miRNA, the PCR reverse primer will be the Universal Adaptor PCR Primer included in the kit

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. The reverse transcribed cDNA is used as template, diluted before use. Do not add more than 5% of the original cDNA solution volume to the total qPCR reaction solution.

f. 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 (refer to the table below).

| Instrument | ROX per 20 µl PCR reaction | Final concentration |
|---|---|---|
| Bio-Rad 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 require calibration using ROX but have not been listed in the table, please optimize the concentration of ROX according to the guidelines of your specific instrument.*

3. Mix the qPCR reaction mix sufficiently and add to the qPCR reaction plates.
4. Briefly centrifuge the plates to make sure all the reagents are at the bottom of each well.
5. The following two-step method for programming the PCR reaction is recommended:

| Cycles | Steps | Temperature | Time | Detection |
|---|---|---|---|---|
| 1 | Initial denaturation | 95°C | 30 sec | No |
| 40 | Denaturation | 95°C | 10 sec | No |
| | Annealing and | 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 (the example was adapted from the ViiA™7 Real Time PCR detection system from Applied Biosystems):

| Step | Temperature range | Heating Rate | Constant temperature/ | Detection |
|---|---|---|---|---|
| 1 | 72–95°C | 2.05°C/sec | 95°C/15 sec | No |
| 2 | 95–60°C | -1.71°C/sec | 60°C/60 sec | No |
| 3 | 60–95°C | 0.05°C/sec | 95°C/15 sec | Yes |

*The conditions for your instrument may differ, please consult the documentation of your qPCR instrument for instructions.*

b. The DNA polymerase used in the 5× BlazeTaq qPCR Mix is a special antibody modified hot-start enzyme. Incubation for 30 seconds at 95°C will sufficiently activate the enzyme.

c. 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 500 bp are acceptable.

d. The principal conditions for the above reaction are referred to in the ViiA7 qPCR instrument manual from ABI. 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.

# V. Limited Use License and Warranty

### Limited Use License

The following terms and conditions apply to use of ExoCt™ 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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