SuperPrep™ II Cell Lysis & RT Kit for qPCR

SCQ-401 100 reactions

Store at -20°C

SuperPrep™ II Cell Lysis Kit for qPCR

SCQ-501 100 preparations

Store at -20°C

Contents

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnostic or clinical purposes. Please observe general laboratory safety precautions while using this kit.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO (SHANGHAI) BIOTECH, CO., LTD.
Tel (+86)-21-58794900

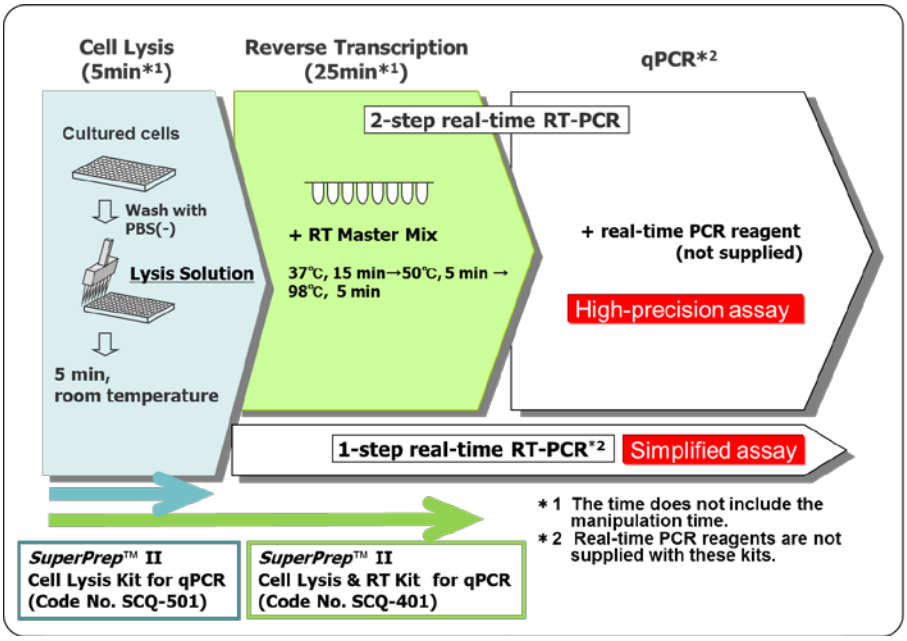
FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

[1] Introduction

Description

SuperPrep™ II Cell Lysis & RT Kit for qPCR (Code No. SCQ-401) consists of "Lysis Reagents" and "RT Reagents" for synthesis of cDNA templates for real-time PCR assays. "Lysis Reagents" prepare cell lysates containing RNAs that can be used as templates for reverse transcription. "RT Reagents" contain reagents for reverse transcription, optimized for efficient cDNA synthesis from crude lysates. The synthesized cDNA can be applied to real-time PCR directly. This assay system is suitable for high-throughput assays. *SuperPrep*™ II can directly move to the cDNA synthesis step after adding lysis solution to cells, whereas the previous version requires Stop Solution after the Lysis step. Moreover, *SuperPrep*™ II realizes stable analysis in a wider range of mamalian cells, including primary cells. With this assay system, it is possible to synthesize template cDNA for real-time PCR from cultured cells conveniently and quickly.

SuperPrep™ II Cell Lysis Kit for qPCR (Code No. SCQ-501) is an option for "Lysis Reagents". The cell lysate prepared by "Lysis Reagents" can be applied to one-step real-time PCR.



Features

-RNA purification is not necessary.

Cell lysates can be prepared as effective templates for cDNA synthesis, reducing the total assay time.

-High-quality cDNA can be obtained from cell lysates.

The optimized lysis solution efficiently inhibits RNA degradation during treatment. RNA in the lysate is stable on ice for at least 6 h. High-quality cDNA can be synthesized using highly efficient reverse transcriptase "ReverTra Ace™" with low contamination of genomic DNA because of preceding DNase I treatment. The reverse transcriptase is supplied as a master mix reagent containing optimally mixed primers (random and oligo dT) to achieve effective cDNA synthesis.

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CHINA
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Table 1 Cells tested by this system

	Cell Name	Adherent/ Non-adherent	Species	Remarks
1	HPA	Adherent	*H. sapiens*	preadipocytes (primary cell)
2	HEK	Adherent	*H. sapiens*	epidermal keratinocytes (primary cell)
3	HA	Adherent	*H. sapiens*	astrocytes (primary cell)
4	HDF	Adherent	*H. sapiens*	dermal fibroblasts (primary cell)
5	HFLS	Adherent	*H. sapiens*	fibroblast-like synoviocytes (primary cell)
6	HBEpC	Adherent	*H. sapiens*	bronchial epithelial cells (primary cell)
7	HUVEC	Adherent	*H. sapiens*	umbilical vein endothelial cells (primary cell)
8	HPAEC	Adherent	*H. sapiens*	pulmonary artery endothelial cells (primary cell)
9	HC	Adherent	*H. sapiens*	chondrocytes (primary cell)
10	HOb	Adherent	*H. sapiens*	osteoblasts (primary cell)
11	HSkMC	Adherent	*H. sapiens*	skeletal muscle cells (primary cell)
12	HAOSMC	Adherent	*H. sapiens*	aortic smooth muscle cells (primary cell)
13	HFDPC	Adherent	*H. sapiens*	hair follicle dermal papilla cells (primary cell)
14	HMSC	Adherent	*H. sapiens*	marrow stromal cell (primary cell)
15	A431	Adherent	*H. sapiens*	epidermoid carcinoma cell line
16	HeLa S3	Adherent	*H. sapiens*	cervix carcinoma cell line
17	HepG2	Adherent	*H. sapiens*	hepatocellular carcinoma cell line
18	C_2C_{12}	Adherent	*M. musculus*	myoblast cell line
19	NIH-3T3	Adherent	*M. musculus*	embryo fibroblast cell line
20	MDCK	Adherent	*C. familiaris*	kidney cell line
21	CHO-K1	Adherent	*C. griseus*	ovary cell line
22	Jurkat	Non-adherent	*H. sapiens*	T lymphocyte cell line
23	K562	Non-adherent	*H. sapiens*	myelogenous leukemia cell line
24	THP-1	Non-adherent	*H. sapiens*	acute monocytic leukemia cell line
25	U937	Non-adherent	*H. sapiens*	leukemic monocyte lymphoma cell line
26	HMNC	Non-adherent	*H. sapiens*	mononuclear cells (primary cell)

-Reduction of dispersion on high-throughput assay

Decreasing the small-volume dispensing and dilution steps from the protocol reduced the dispersion of data in a high-throughput assay. Integrating or omitting some steps improved the usability. Cells can be lysed on the culture plate without pipetting, and DNase I treatment can be performed at the same time as the lysis step. Additional stop solution, purification or heating steps are unnecessary.

-Various real-time PCR reagents can be applied

The synthesized cDNA can be used in various real-time PCR assays (TaqMan® probe, SYBR® Green etc). The examples using THUNDERBIRD™ qPCR Mix (Code No. QPS-101, QPS-201) and KOD SYBR™ qPCR Mix (Code No. QKD-201) are detailed in [4] 1.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO (SHANGHAI) BIOTECH, CO., LTD.
Tel (+86)-21-58794900

In addition, the cell lysate can be applied to one-step real-time PCR reagents. The examples using *RNA-direct*™ real-time PCR Master Mix (Code No. QRT-101, 201) are detailed in [4] 2.

[2] Components

The kits include the following reagents, which can be used for 100 (SCQ-401), 20 (SCQ-401S) and 100 (SCQ-501) reactions. SCQ-401 and SCQ-401S contain two separate packages named “Lysis Reagents” and “RT Reagents”, respectively. All reagents should be stored at -20°C.

<u>*SuperPrep*™ II Cell Lysis & RT Kit for qPCR (Code No. SCQ-401, SCQ-401S)</u>

<Lysis Reagents>	SCQ-401	SCQ-401S (SAMPLE)
Lysis Solution*1	6.5 mL	1.3 mL
gDNA Remover	33 µL	6.6 µL
RNase Inhibitor	110 µL	22 µL

<RT Reagents*2>		
5 × RT Master Mix	860 µL	172 µL
5 × RT Master Mix no-RT Control	86 µL	17 µL
Nuclease-free Water	1.7 mL × 1	680 µL

<u>*SuperPrep*™ II Cell Lysis Kit for qPCR (Code No. SCQ-501)</u>

	SCQ-501
Lysis Solution*1	6.5 mL
gDNA Remover	33 µL
RNase Inhibitor	110 µL

*1 Lysis Solution can be stored at 2–8°C for 5 months. However, Lysis Solution contains gDNA Remover and RNase Inhibitor, and should not be stored.
*2 For extended storage of “RT Reagents”, -30°C is recommended.

Lysis Solution

Lysis Solution should be used with gDNA Remover and RNase Inhibitor to protect RNA in the cell lysate. The solution also minimizes the inhibition of cDNA synthesis by cell components.

gDNA Remover

gDNA remover is an optimized DNase I solution to degrade contaminated gDNAs in the cell lysate. gDNA Remover should be used with Lysis Solution.

RNase Inhibitor

RNase Inhibitor should be used with Lysis Solution to inhibit RNase in the cell lysate.

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Tel (+86)-21-58794900

5× RT Maser Mix

This reagent is a 5× master mix that contains highly efficient reverse transcriptase "ReverTra Ace™", RNase Inhibitor, oligo dT primer, random primer and dNTPs.

5× RT Maser Mix no-RT Control

The composition of "5× RT Master Mix no-RT Control" is identical to that of "5× RT Master Mix," except that the reverse transcriptase (RT) is omitted. This master mix can be used in control experiments because of the absence of reverse transcriptase.

Nuclease-free Water

This nuclease-free water has been prepared without DEPC treatment.

[3] Protocol

1. Preparation of cell lysates

(1) For adherent cells cultured in a 96-well culture plate

a. Seed an appropriate number of cells in a 96-well culture plate.

Notes

Lysis Solution can treat 1×10^{1}–7×10^{4} cells. However, excessive cells cause an insufficient lysis, inhibition of RT-PCR and partial digestion of genomic DNA. The cell number for treatment should be determined by a preliminary experiment when treating $>10^{4}$ cells.

b. Culture and incubate the cells.

c. Remove the medium from the wells.

d. Add 100 µL of PBS(-) to the wells.

e. Remove PBS(-) from the wells.

f. [Preparation of Lysis Solution with gDNA Remover and RNase Inhibitor]

Add 0.3 µL gDNA Remover and 1 µL RNase Inhibitor to 58.7 µL Lysis Solution. The mixture can be prepared as one solution according to sample numbers. When using a multi-channel pipet, the amount of the mixture should be increased by about 10% because of waste.

Notes

Do not store the mixed solution.

g. Add 60 µL Lysis Solution with gDNA Remover and RNase Inhibitor to wells.

h. Mix the plate gently using a plate mixer or tapping with hands for 30 sec and incubate at room temperature for 4.5 min.

Notes

The incubation time can be increased up to 10 min. The lysate can be stored on ice up to 6 h.

i. Transfer the plate to ice.

Notes
-cDNA should be synthesized using the lysate as soon as possible, although the stability of the RNA in the lysate has been confirmed as at least 6 h. The lysate should be stored at -80°C for 2 months.
-The synthesized cDNA can be stored stably.

(2) For non-adherent cells cultured in a 96-well culture plate

a. Seed an appropriate number of cells in a 96-well culture plate.

Notes
Lysis Solution can treat 1×10^{1}–7×10^{4} cells. However, excessive cells cause an insufficient lysis, inhibition of RT-PCR and partial digestion of genomic DNA. The cell number for treatment should be determined by a preliminary experiment when treating $>10^{4}$ cells. The cell number for the assay should be adjusted by removing an appropriate number of cells from wells.

b. Culture and incubate the cells.

c. Centrifuge the plate at 2,000 rpm for 5 min using a plate centrifuge.

d. Remove the medium.

Notes
The medium should be removed by pipetting or aspiration. Decantation is not recommended.

e. Add 100 µL of PBS(-).

Notes
Pipetting is not necessary.

f. Centrifuge the plate at 2,000 rpm for 5 min using a plate centrifuge.

g. Remove the PBS(-).

h. Go to [3] 1.(1) f.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
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CHINA
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Tel (+86)-21-58794900

(3) For cells cultured in plates (except 96-well plates)

If using anything other than a 96-well plate, refer to Table 1 below for the number of cells to seed per well and the volumes of PBS(-) and Lysis Solution to add per well.

Table 1 Cell Number and Buffer Volumes for Different Plate Formats

Plate format	Number of cells to seed per well	Volume of PBS(-) to add per well	Volume of Lysis Solution[*1] to add per well
384-well plate	$4x10^3$ cells	100µl	16µl
48-well plate	$2x10^4$ cells	250µl	120µl
24-well plate	$4x10^4$ cells	500µl	240µl
12-well plate	$8x10^4$ cells	1000µl	480µl
6-well plate	$1x10^5$ cells	2000µl	960µl

[*1] Includes gDNA Remover and RNase Inhibitor.

(4) For harvested cells

a. Harvest the cells into a centrifuging tube and count the cell number.

b. Pellet the cells and suspend the cells in PBS(-).

c. Adjust the cell density to < 1×10^7 cells/mL by PBS(-).

d. Dispense 5 µL of cell suspension into the wells.

e. Go to [3] 1.(1) f.

2. Reverse transcription

(1) [Preparation of reaction mixture (40 µL reaction)]

Add 8 µL 5×RT Master Mix to 24 µL Nuclease-free water in a tube.
The mixture can be prepared as one solution according to sample numbers. When using a multi-channel pipet, the amount of the mixture should be increased by about 10% because of waste.

Notes

For the negative control experiments without the reverse transcriptase, 5× RT Master Mix no-RT Control can be used. The kit (Code No.: SCQ-401) contains sufficient no-RT control for 10 reactions.

(2) Dispense 32 µL reaction mixtures into a PCR plate.

(3) Add 8 µL of the lysate [from [3] 1.(1) i.] and mix gently.

(4) Spin down the solutions.

(5) Incubate the mixture as follows:

37°C, 15 min[*1]
50°C, 5 min
98°C, 5 min
4°C, Hold

[*1] The incubation time can be increased up to 60 min.

(6) Go to the real-time PCR step.

Notes
The synthesized cDNA can be stored at 4°C for a week and -20°C long term.

[4] Real-time PCR

1. For assays using two-step real-time PCR reagents

The synthesized cDNA (from [3]2,(6)) can be applied to various kinds of real-time PCR assay directly. Ten to 15% (v/v) cDNA can be added to the PCR reaction to achieve good reproducibility. The optimal amount of cDNA should be determined by preliminary tests. The conditions for several real-time PCR reagents are shown below as examples.

(1) THUNDERBIRD™ Probe qPCR Mix (Code No. QPS-101) for TaqMan® assay
The detailed conditions should be referred to in the instruction manual of THUNDERBIRD™ Probe qPCR Mix (Code No. QPS-101).

a. Reaction setup

Reagent	Reaction volume 50 µl	20 µl	Final concentration
DW	X µl	X µl	
THUNDERBIRD™ Probe qPCR Mix	25 µl	10 µl	1×
Forward primer	15 pmol	6 pmol	0.3 µM
Reverse primer	15 pmol	6 pmol	0.3 µM
TaqMan® Probe	10 pmol	4 pmol	0.2 µM[*1]
50× ROX reference dye	1 µl / 0.1 µl	0.4 µl / 0.04 µl	1× / 0.1×[*2]
cDNA solution	~7.5 µl	~3 µl	
Total	50 µl	20 µl	

[*1] 50× ROX reference dye must be added when using real-time cyclers that require a passive reference dye, according to Table 1. Table 1 shows the optimum concentration of the ROX reference dye. This dye is not necessary for real-time cyclers that do not require a passive reference dye.

Table 1 Recommended ROX dye concentration

Real-time cycler	Optimal dye concentration (dilution ratio)
Applied Biosystems 7000, 7300, 7700, 7900HT StepOne™, StepOnePlus™, etc.	1× (50:1)
Applied Biosystems 7500, 7500Fast, Agilent cyclers (Optional), etc.	0.1× (500:1)
Roche cyclers, Bio-Rad cyclers, BioFlux cyclers, etc.	Not required

b. PCR cycling condition

The following table shows the recommended thermal conditions using primers designed according to the recommended primer and probe conditions described in the instruction manual of THUNDERBIRD™ Probe qPCR Mix (Code No. QPS-101).

<Two-step cycle>	Temperature	Time	Ramp	
Pre-denaturation:	95°C	60 sec[*1]	Maximum	
Denaturation:	95°C	15 sec[*2]	Maximum	↰ 40 cycles
Extension:	60°C	60 sec	Maximum	
	(data collection should be set at the extension step)			

[*1] In the anti-Taq antibody hot start PCR system, the pre-denaturation can be completed within 60 sec. The pre-denaturation time should be determined according to the recommendations of each real-time cycler. If the optimal pre-denaturation time cannot be determined, the time should be set at 60 sec.

Table 2 The recommended pre-denaturation time for each real-time cycler

Real-time cycler	Pre-denaturation time
High-speed cycler (e.g. Applied Biosystems 7500Fast)	20 sec
Capillary cycler (e.g. Roche LightCycler™ 1.x, 2.0)	30 sec
General real-time cyclers (e.g. Applied Biosystems 7700, 7500, 7900HT [normal block], StepOne™, StepOnePlus™ Agilent cyclers, BioFlux cyclers, Bio-Rad CFX, QIAGEN Rotor-Gene)	60 sec

[*2] The following table shows the optimal denaturation times for each real-time cycler. If the optimal denaturation time cannot be determined, the time should be set at 15 sec.

Table 3 The recommended denaturation time for each real-time cycler

Real-time cycler	Denaturation time
High-speed cycler (e.g. Applied Biosystems 7500Fast)	3 sec
Capillary cycler (e.g. Roche LightCycler™ 1.x, 2.0)	5 sec
General real-time cyclers (e.g. Applied Biosystems 7700, 7500, 7900HT [normal block], StepOne™, StepOnePlus™ Agilent cyclers, BioFlux cyclers, Bio-Rad CFX, QIAGEN Rotor-Gene)	15 sec

JAPAN
TOYOBO CO., LTD.
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tech_osaka@toyobo.jp

CHINA
TOYOBO (SHANGHAI) BIOTECH, CO., LTD.
Tel (+86)-21-58794900

(2) KOD SYBR® qPCR Mix (Code No. QKD-201) for SYBR® Green I assay

The detailed conditions should be referred to in the instruction manual of KOD SYBR® qPCR Mix (Code No. QKD-201).

a. Reaction setup

	Reaction volume		Final
Reagent	50 µl	20 µl	concentration
DW	X µl	X µl	
KOD SYBR® qPCR Mix	25 µl	10 µl	1×
Forward primer	10 pmol	4 pmol	0.2 µM
Reverse primer	10 pmol	4 pmol	0.2 µM
50× ROX reference dye	1 µl / 0.1 µl	0.4 µl / 0.04 µl	1× / 0.1×[*1]
cDNA solution	~7.5 µl	~3 µl	
Total	50 µl	20 µl	

[*1] 50× ROX reference dye must be added when using real-time cyclers that require a passive reference dye, according to Table 1. Table 1 shows the optimum concentration of the ROX reference dye. This dye is not necessary for real-time cyclers that do not require a passive reference dye.

Table 1 Recommended ROX dye concentration

Real-time cycler	Optimal dye concentration (dilution ratio)
Applied Biosystems 7000, 7300, 7700, 7900HT StepOne™, StepOnePlus™, etc.	1× (50:1)
Applied Biosystems 7500, 7500Fast, Agilent cyclers (Optional), etc.	0.1× (500:1)
Roche cyclers, Bio-Rad cyclers, BioFlux cyclers, etc.	Not required

b. PCR cycling conditions

The following table shows the recommended thermal conditions using primers designed according to the recommended primer conditions.

<Two-step cycle>	Temperature	Time	Ramp	
Pre-denaturation:	95°C	60 sec[*1]	Maximum	
Denaturation:	95°C	15 sec[*2]	Maximum	↰ 40 cycles
Extension:	60°C	60 sec	Maximum	
	(data collection should be set at the extension step)			
	Melting / Dissociation Curve Analysis[*3]			

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TOYOBO CO., LTD.
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tech_osaka@toyobo.jp

CHINA
TOYOBO (SHANGHAI) BIOTECH, CO., LTD.
Tel (+86)-21-58794900

[*1] In the anti-Taq antibody hot start PCR system, the pre-denaturation can be completed within 60 sec. The pre-denaturation time should be determined according to the recommendations of each real-time cycler. If the optimal pre-denaturation time cannot be determined, the time should be set at 60 sec.

Table 2 The recommended pre-denaturation time for each real-time cycler

Real-time cycler	Pre-denaturation time
High-speed cycler (e.g. Applied Biosystems 7500Fast)	20 sec
Capillary cycler (e.g. Roche LightCycler™ 1.x, 2.0)	30 sec
General real-time cyclers (e.g. Applied Biosystems 7700, 7500, 7900HT [normal block], StepOne™, StepOnePlus™ Agilent cyclers, BioFlux cyclers, Bio-Rad CFX, QIAGEN Rotor-Gene)	60 sec

[*2] The following table shows the optimal denaturation times for each real-time cycler. If the optimal denaturation time cannot be determined, the time should be set at 15 sec.

Table 3 The recommended denaturation time for each real-time cycler

Real-time cycler	Denaturation time
High-speed cycler (e.g. Applied Biosystems 7500Fast)	3 sec
Capillary cycler (e.g. Roche LightCycler™ 1.x, 2.0)	5 sec
General real-time cyclers (e.g. Applied Biosystems 7700, 7500, 7900HT [normal block], StepOne™, StepOnePlus™ Agilent cyclers, BioFlux cyclers, Bio-Rad CFX, QIAGEN Rotor-Gene)	15 sec

[*3] Detailed conditions for the melting curve analysis should be referred to in the instruction manuals of the real-time PCR reagents.

(3) THUNDERBIRD™ SYBR® qPCR Mix (Code No. QPS-201) for SYBR® Green I assay

The detailed conditions should be referred to in the instruction manual of THUNDERBIRD™ SYBR® qPCR Mix (Code No. QPS-201).

a. Reaction setup

	Reaction volume		Final
Reagent	50 µl	20 µl	concentration
DW	X µl	X µl	
THUNDERBIRD™ SYBR® qPCR Mix	25 µl	10 µl	1×
Forward primer	15 pmol	6 pmol	0.3 µM
Reverse primer	15 pmol	6 pmol	0.3 µM
50× ROX reference dye	1 µl / 0.1 µl	0.4 µl / 0.04 µl	1× / 0.1×[*1]
cDNA solution	~5 µl	~2 µl	
Total	50 µl	20 µl	

*1 See [4]1(2) Table 1.

b. PCR cycling conditions
See [4]1(2) b.

(3) Other real-time PCR reagents

The synthesized cDNA (from [3]2(6)) can be applied to various kinds of real-time PCR assay directly. Ten to 15% (v/v) cDNA can be added to the PCR reaction to achieve good reproducibility. The optimal amount of cDNA should be determined by preliminary tests. The detailed conditions should be set for each real-time PCR reagent.

Notes

In the case of real-time PCR reagents with hot start technology based on chemical modification, the cDNA should be added up to 10% (v/v) in the PCR reaction.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO (SHANGHAI) BIOTECH, CO., LTD.
Tel (+86)-21-58794900

2. For assays using one-step real-time PCR reagents

The cell lysates (from [3]1(1) i) can be applied to various kinds of one-step real-time PCR assay directly. The optimal amount of cDNA should be determined by preliminary tests. The conditions for several one-step real-time PCR reagents are shown below as an example.

(1) *RNA-direct*™ Real-time PCR Master Mix (Code No. QRT-101) for TaqMan® assay.

The detailed conditions should be referred to in the instruction manual of *RNA-direct*™ Real-time PCR Master Mix (Code No. QRT-101).

a. Reaction setup

	Reaction volume		Final
Reagent	50 µl	20 µl	concentration
DW	X µl	X µl	
RNA-direct™ Real-time PCR Master Mi	25 µl	10 µl	1×
50 mM $Mn(OAc)_2$	2.5 µl	1 µl	2.5 mM
Forward primer	15 pmol	6 pmol	0.3 µM
Reverse primer	15 pmol	6 pmol	0.3 µM
TaqMan® Probe	10 pmol	4 pmol	0.2 µM
Cell lysate ([3]1(1) l)	2.5–5 µl	1–2 µl	
Total	50 µl	20 µl	

b. PCR cycling condition

The following table shows the recommended thermal conditions using primers designed according to the recommended primer and probe conditions described in the instruction manual of *RNA-direct*™ Real-time PCR Master Mix (Code No. QRT-101) for TaqMan® assay.

<Two-step cycle>	Temperature	Time	Ramp	
Pre-denaturation:	90°C	30 sec	Maximum	
Reverse transcription:	55°C	20 sec	Maximum	
Denaturation:	95°C	60 sec	Maximum	
Denaturation	95°C	15 sec	Maximum	] 40 cycles
Extension:	60°C	60 sec	Maximum	
	(data collection should be set at the extension step)			

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
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tech_osaka@toyobo.jp

CHINA
TOYOBO (SHANGHAI) BIOTECH, CO., LTD.
Tel (+86)-21-58794900

Probe One-step qRT-PCR Kit (Code No. QRZ-101) for TaqMan® assay.

The detailed conditions should be referred to in the instruction manual of THUNDERBIRD™ Probe One-step qRT-PCR Kit (Code No. QRZ-101).

a. Reaction setup

	Reaction volume		Final
	50 µl	20 µl	Concentration
RNase free water	X µl	X µl	
2× Reaction buffer	25 µl	10 µl	1×
DNA Polymerase	1.25 µl	0.5 µl	
RT Enzyme Mix	1.25 µl	0.5 µl	
Forward primer	25 pmol	10 pmol	0.5 µM
Reverse primer	25 pmol	10 pmol	0.5 µM
TaqMan® probe	10 pmol	4 pmol	0.2 µM
50× ROX Reference dye	1µl / 0.1 µl	0.4 µl / 0.04 µl	1× / 0.1×*1
(Uracil-N-glycosylase)*2 [optional]	1 unit	0.4 units	1 unit / 50 µl
Cell lysate ([3]1(1) l)	2.5~5 µl	1~2 µl	
	50 µl	20 µl	

*1 50× ROX Reference dye must be added when using real-time cyclers that require a passive reference dye, according to Table 1. Table 1 shows the optimum concentration of the ROX Reference dye. This dye is not necessary for real-time cyclers that do not require a passive reference dye.

*2 Heat-labile uracil-N-glycosylase (UNG) should be used. The amount of UNG can be changed according to the recommendations from each supplier. UNG is not supplied with this kit.

Table 1 Recommended ROX dye concentration

Real-time cycler	Optimal dye concentration (dilution ratio)
Applied Biosystems® 7000, 7300, 7700, 7900HT StepOne™, StepOnePlus™, etc.	1× (50:1)
Applied Biosystems® 7500, 7500Fast, Agilent cyclers (Optional), etc.	0.1× (500:1)
Roche cyclers, Bio-Rad cyclers, Qiagen cyclers, etc.	Not required

b. PCR cycling condition

The following table shows the recommended thermal conditions using primers designed according to the recommended primer and probe conditions described in the instruction manual of THUNDERBIRD™ Probe One-step qRT-PCR Kit (Code No. QRZ-101) for TaqMan® assay.

(2) THUNDERBIRD™

(UNG treatment)	(20–25°C[*1])	(10 min[*1])	(Maximum)	
Reverse transcription	50°C	10 min	Maximum	
Pre-denaturation	95°C	60 s	Maximum	
Denaturation	95°C	15 s	Maximum	40 cycles[*2]
Extension (annealing)	60°C	45 s	Maximum	
	(Data collection should be performed at the extension step.)			

*1 [Optional] The uracil-N-glycosylase (UNG) treatment step should be added before the cDNA synthesis step. The indicated temperature and time are typical conditions for UNG. The conditions can be optimized according to the particular instruction manual from the supplier of UNG.

*2 The number of cycles can be increased up to 45.

(3) *RNA-direct*™ SYBR® Green Real-time PCR Master Mix (Code No. QRT-201) for SYBR® Green I assay.

The detailed conditions should be referred to in the instruction manual of *RNA-direct*™ SYBR® Green Real-time PCR Master Mix (Code No. QRT-201).

a. Reaction setup

	Reaction volume		Final
Reagent	50 µl	20 µl	concentration
DW	X µl	X µl	
RNA-direct™ SYBR® Green Real-time PCR Master Mix	25 µl	10 µl	1×
50 mM $Mn(OAc)_2$	2.5 µl	1 µl	2.5 mM
Forward primer	10 pmol	4 pmol	0.2 µM
Reverse primer	10 pmol	4 pmol	0.2 µM
Cell lysate ([3]1(1) l)	2.5–5 µl	1–2 µl	
Total	50 µl	20 µl	

b. PCR cycling condition

The following table shows the recommended thermal conditions using primers designed according to the recommended primer conditions described in the instruction manual of *RNA-direct*™ SYBR® Green Real-time PCR Master Mix (Code No. QRT-201).

	Temperature	Time	Ramp

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Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
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Tel (+86)-21-58794900

Pre-denaturation:	90°C	30 sec	Maximum	
Reverse transcription:	55°C	20 sec	Maximum	
Denaturation:	95°C	60 sec	Maximum	↰
Annealing	55°C	15 sec	Maximum	] 40 cycles
Extension:	74°C	60 sec	Maximum	
	(data collection should be set at the extension step)			
	Melting / Dissociation Curve Analysis			

<Three-step cycle>	Temperature	Time	Ramp

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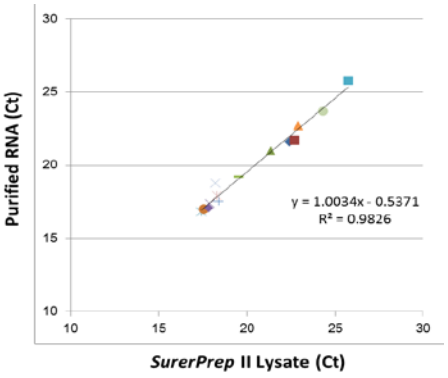
[5] Application data

1. Comparison of the efficiency with purified RNA

SuperPrep™ II Cell Lysis & RT Kit for qPCR (Code No SCQ-401) synthesized cDNA using the lysate from 2.5×10^4 HUVEC in a 40 µL reaction. cDNA was synthesized using 66.6 ng of total RNA, corresponding to 2.5×10^4 HUVEC, using a cDNA synthesis kit (ReverTra Ace™ qPCR RT Master Mix [Code No. FSQ-201]) in a 40 µL reaction. Fifteen housekeeping genes were then analyzed by SYBR® Green real-time PCR using the synthesized cDNA.

High correlation was observed between the two methods. The method using *SuperPrep*™ II facilitated the expression analysis by real-time PCR without time-consuming RNA purification steps.



2. Evaluation of the assay variation

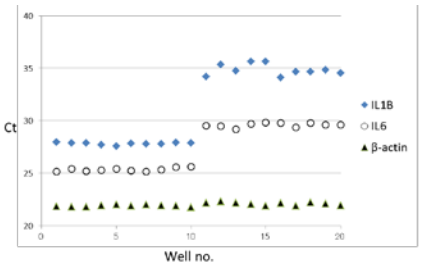
HeLa S3 cells were incubated with or without 100 nM phorbol 12-myristate 13-acetate (PMA) for 24 h after seeding at 2×10^4 cells/well in a 96-well culture plate. cDNA were synthesized from the lysates prepared from the cells washed with PBS(-).IL-6, IL-1β and β-actin genes were detected by TaqMan® real-time PCR assay with THUNDERBIRD™ Probe qPCR Mix (Code No. QPS-101). After compensation of the Cts of IL-6 and IL-1β by that of β-actin, the ΔΔCt between with or without PMA and Z' factors[*1] were calculated.

Z' factors from *SuperPrep*™ II were superior to that from the other system (Company A).

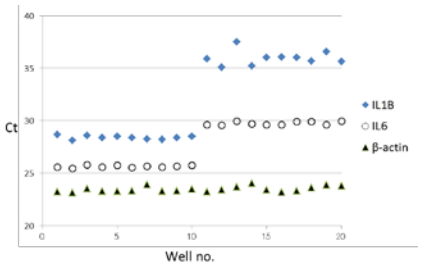
[*1] The Z' factor is a simple statistical parameter that is used to assess the quality of high-throughput screening (HTS) assays. A Z' score of 0.5 is generally considered to indicate good quality Z' can be calculated by the following formula.

$$Z' = 1\text{-}3 \times [\Delta Ct(+) \text{ standard deviation} + \Delta Ct(-) \text{ standard deviation}] / |\Delta\Delta Ct|$$

SuperPrep™ II



Company A



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IL-6	PMA	Ct (IL-6) Mean	ΔCt (IL-6-β-actin) Mean	S.D.	ΔΔCt	Z'
SuperPrep™ II	(+)	25.35	3.45	0.20	-4.05	0.66
	(-)	29.60	7.50	0.26		
Company A	(+)	25.64	2.24	0.19	-3.94	0.64
	(-)	29.76	6.18	0.29		

IL-1β	PMA	Ct (IL-1β) Mean	ΔCt (IL-1β-β-actin) Mean	S.D.	ΔΔCt	Z'
SuperPrep™ II	(+)	27.83	5.93	0.18	-6.81	0.66
	(-)	34.85	12.75	0.59		
Company A	(+)	28.42	5.01	0.28	-7.38	0.59
	(-)	35.98	12.39	0.74		

3. Stability test of cell lysates

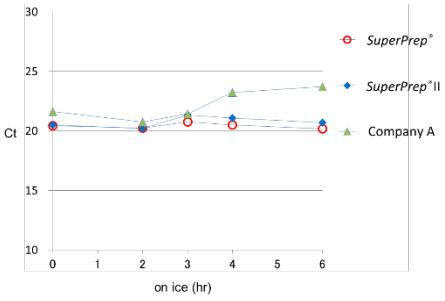
cDNA were synthesized from lysates that had been left on ice for 0–6 h after lysing of 4×10^4 HeLa and U937 cells using *SuperPrep*™ II. β-actin genes were detected using TaqMan® real-time PCR assay with THUNDERBIRD™ Probe qPCR Mix (Code No. QPS-101). The results were compared with that from the other company's system (Company A).

The results suggest that the RNA in the cell lysates is stable for at least 6 h.

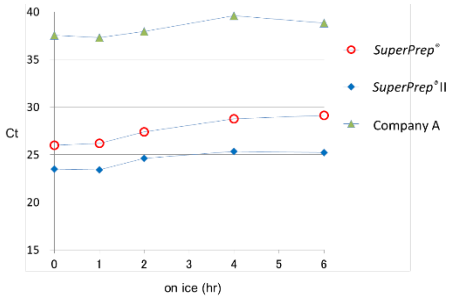
Notes

RNase activity depends on the type and number of cells. The cell lysates should be placed on ice after preparation and cDNA should be synthesized immediately after preparing the lysates to minimize RNA degradation.

HeLa S3



U937



[6] Troubleshooting

Symptom	Cause	Solution
No or low signal on real-time PCR	Excessive number of cells on lysate preparation	Excessive cellular compounds may inhibit cDNA synthesis and qPCR steps. Decrease the number of cells in the lysate preparation or dilute the cell lysates by the same solution before cDNA synthesis.
	Degradation of RNA	-cDNA should be synthesized immediately after preparing the cell lysates, especially on the cells exhibiting high RNase activity. (Cell lysates from general cells can be stored stably on ice for at least 6 h.) -The cell lysates should be stored at -80°C avoiding freezing and thawing. -Harvested cells should be stored at -80°C after washing PBS(-).
	Excess amount of cDNA solution compared with the total PCR reaction volume	Ten to 15% (v/v) cDNA solution can be added to the PCR reaction. However, tolerance of the amount of the cDNA solution in the reaction may vary. The optimal amount of cDNA should be determined by preliminary tests. The conditions for several real-time PCR reagents are shown below as examples.
Low yield	Cell lysis step was insufficient	Mix thoroughly after adding Lysis Solution.

[7] Related products

Product name	Package	Code No.
Real-time PCR master mix for probe assay **THUNDERBIRD™ Probe qPCR Mix**	1.67 mL × 3	QPS-101
Real-time PCR master mix for SYBR® Green assay **KOD SYBR® qPCR Mix**	1.67 mL × 3	QKD-201
Real-time PCR master mix for SYBR® Green assay **THUNDERBIRD™ SYBR® qPCR Mix**	1.67 mL × 3	QPS-201
One-step Real-time PCR master mix for probe assay **THUNDERBIRD™ Probe One-step qRT-PCR Kit**	1.25 mL × 2	QRZ-101
One-step Real-time PCR master mix for probe assay ***RNA-direct*™ Real-time PCR Master Mix**	0.5 mL × 5	QRT-101
One-step Real-time PCR master mix for SYBR® Green assay ***RNA-direct*™ SYBR® Green Realtime PCR Master Mix**	0.5 mL × 5	QRT-201

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