SuperPrep™ Cell Lysis & RT Kit for qPCR

SCQ-101 100 reactions

Store at -20°C

SuperPrep™ Cell Lysis Kit for qPCR

SCQ-201 100 preparations

Store at -20°C

Contents

[1] **Introduction**

[2] **Components**

[3] **Protocol**

1. Preparation of cell lysate
2. Reverse transcription

[4] **Real-time PCR**

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

[5] **Application data**

[6] **Troubleshooting**

[7] **Related products**

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.

-LightCycler™ is a trademark of Idaho Technology, Inc. and Roche Molecular Systems, Inc.
-SYBR® is a registered trademark of Molecular Probes, Inc.
-TaqMan® is a registered trademark of Roche Molecular Systems, Inc.

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

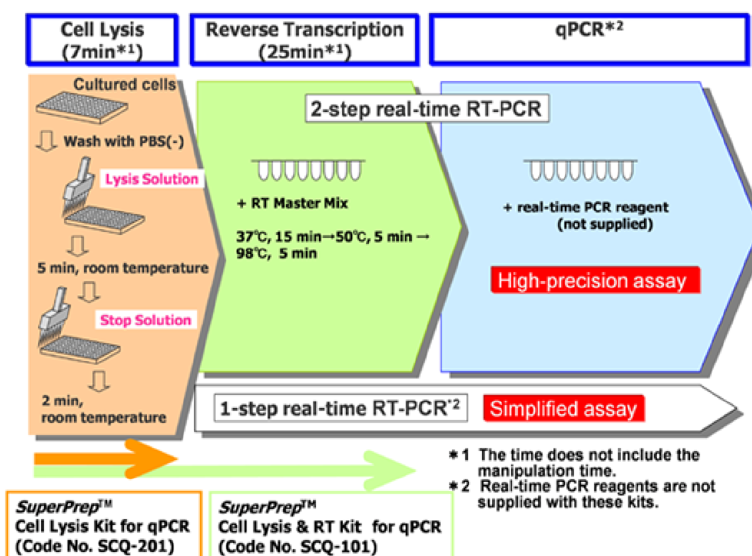
FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

[1] Introduction

Description

SuperPrep™ Cell Lysis & RT Kit for qPCR (Code No. SCQ-101) consists of "Lysis Reagents" and "RT Reagents" for synthesis of cDNA templates for real-time PCR assays. "Lysis Reagents" prepares cell lysates containing RNAs that can be used as templates for reverse transcription. "RT Reagents" contains 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™ Cell Lysis Kit for qPCR (Code No. SCQ-201) is an option of "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 2 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.

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.

Table 1 Cells tested by this system

	Cell Name	Adherent/ Non-adherent	Species	Remarks
1	A431	Adherent	*H. sapiens*	epidermoid carcinoma cell line
2	C_2C_{12}	Adherent	*M. musculus*	myoblast cell line
3	Caco-2	Adherent	*H. sapiens*	colon adenocarcinoma cell line
4	CHO-K1	Adherent	*C. griseus*	ovary cell line
5	COLO205	Non-adherent	*H. sapiens*	colon adenocarcinoma cell line
6	DLD-1	Adherent	*H. sapiens*	colon adenocarcinoma cell line
7	HCT-15	Adherent	*H. sapiens*	colon adenocarcinoma cell line
8	HDF	Adherent	*H. sapiens*	primary foreskin fibroblasts (primary cell)
9	HEK293	Adherent	*H. sapiens*	embryonic kidney cell line
10	HeLa S3	Adherent	*H. sapiens*	cervix carcinoma cell line
11	HepG2	Adherent	*H. sapiens*	hepatocellular carcinoma cell line
12	HUVEC	Adherent	*H. sapiens*	umbilical vein endothelial cells (primary cell)
13	Jurkat	Non-adherent	*H. sapiens*	T lymphocyte cell line
14	K562	Non-adherent	*H. sapiens*	myelogenous leukemia cell line
15	KUSA-A1	Adherent	*M. musculus*	bone marrow stromal stem cell line
16	L929	Adherent	*M. musculus*	aneuploid fibrosarcoma cell line
17	MCF7	Adherent	*H. sapiens*	breast adenocarcinoma cell line
18	Neuro2a	Adherent	*M. musculus*	neuroblastoma cell line
19	NIH-3T3	Adherent	*M. musculus*	embryo fibroblast cell line
20	PC12	Adherent	*R. norvegicus*	adrenal pheochromocytoma cell line
21	rMSC	Adherent	*R. norvegicus*	bone marrow stromal stem cells (primary cell)
22	THP-1	Non-adherent	*H. sapiens*	acute monocytic leukemia cell line
23	U937	Non-adherent	*H. sapiens*	leukemic monocyte lymphoma cell line

-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. The DNase I reaction can be stopped by adding "Stop Solution". Additional purification or heating steps are not necessary.

-Various real-time PCR reagents can be applied

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

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.

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

[2] Components

The kits include the following reagents, which can be used for 100 (SCQ-101), 20 (SCQ-101S), 100 (SCQ-201) and 20 (SCQ-201S) reactions. SCQ-101 and SCQ-101S contain two separate packages named "Lysis Reagents" and "RT Reagents", respectively. All reagents should be stored at -20°C.

***SuperPrep*™ Cell Lysis & RT Kit for qPCR (Code No. SCQ-101, SCQ-101S)**

<Lysis Reagents>	SCQ-101	SCQ-101S (SAMPLE)
Lysis Solution*1	5.5 mL	1.1 mL
gDNA Remover	33 µL	6.6 µL
Stop Solution	1.1 mL	220 µL
RNase Inhibitor	55µL	11µL
<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 × 2	680 µL

***SuperPrer*™ Cell Lysis Kit for qPCR (Code No. SCQ-201, SCQ-201S)**

	SCQ-201	SCQ-201S (SAMPLE)
Lysis Solution*1	5.5 mL	1.1 mL
gDNA Remover	33 µL	6.6 µL
Stop Solution	1.1 mL	220 µL
RNase Inhibitor	55 µL	11 µL

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

Lysis Solution

Lysis Solution should be used with gDNA Remover 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.

Stop Solution

Stop Solution contains components that inhibit enzymes in the cell lysate.

RNase Inhibitor

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

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]

Add 0.3 μL gDNA Remover to 49.7 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 50 μL Lysis Solution with gDNA Remover 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 lysate can be stored on ice up to 2 h.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyoba.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.

i. [Preparation of Stop Solution with RNase Inhibitor]
Add 0.5 μL RNase Inhibitor to 9.5 μL Stop 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 about 10% because of waste.

j. Add 10 μL Stop Solution with RNase Inhibitor to wells.

k. Mix the plate gently using a plate mixer or tapping with hands for 30 sec and incubate at room temperature for 1.5 min.

Notes
In the case of cells that exhibit high RNase activity, the lysate should be incubated on ice to obtain better reproducibility.

l. 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 2 h. The lysate should be stored at -80°C for 2 weeks.
-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.

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.

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.

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

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-101) 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) l.] and mix gently.

(4) Spin down the solutions.

(5) Incubate the mixture as follows:

37°C, 15 min.
50°C, 5 min.
98°C, 5 min.
4°C, Hold

(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	Reaction volume 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)	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)	15 sec.

(2) 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

Reagent	Reaction volume 50 μL	20 μL	Final 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	~7.5 μL	~3μL	
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 and probe conditions described in the instruction manual of THUNDERBIRD™ SYBR® qPCR Mix (Code No. QPS-201).

<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]			

[*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)	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)	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) 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.

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

The cell lysates (from [3]1(1) l) 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

Reagent	Reaction volume 50 μL	20 μL	Final 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)			

(2) *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

Reagent	Reaction volume 50 μL	20 μL	Final 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).

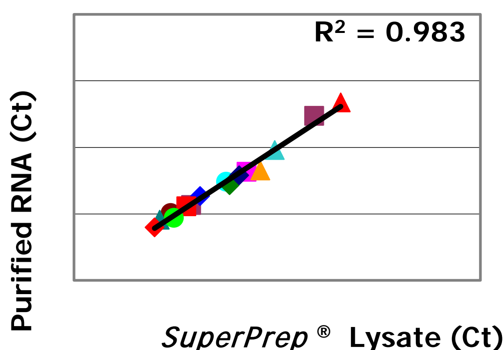
<Three-step cycle>	Temperature	Time	Ramp	
Pre-denaturation:	90°C	30 sec.	Maximum	
Reverse transcription:	55°C	20 sec.	Maximum	
Denaturation:	95°C	60 sec.	Maximum	40 cycles
Annealing	55°C	15 sec.	Maximum	40 cycles
Extension:	74°C	60 sec.	Maximum	40 cycles
	(data collection should be set at the extension step)			
	Melting / Dissociation Curve Analysis			

[5] Application data

1. Comparison of the efficiency with purified RNA

SuperPrep™ Cell Lysis & RT Kit for qPCR (Code No SCQ-101) synthesized cDNA using the lysate from 2.5 × 10^4 HEK293 cells in a 40 μL reaction. cDNA was synthesized using 66.6 ng of total RNA, corresponding to 2.5 × 10^4 HEK293 cells, 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*™ facilitated the expression analysis by real-time PCR without time-consuming RNA purification steps.



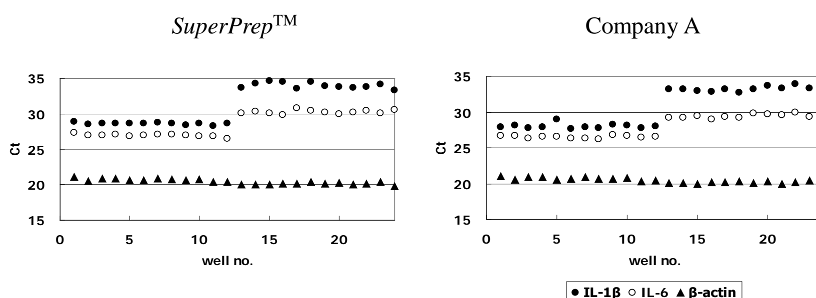
2. Evaluation of the assay variation

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*™ 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'= 1-3 x [Δ Ct(+) standard deviation + Δ Ct(-) standard deviation]/| Δ Δ Ct|



IL-6	PMA	Ct (IL-6) Mean	ΔCt (IL-6-β-actin) Mean	S.D.	ΔΔCt	Z'
SuperPrep™	(+)	26.96	6.27	0.14	-3.85	0.62
	(-)	30.24	10.12	0.35		
Company A	(+)	26.50	4.30	0.34	-3.88	0.59
	(-)	29.44	8.18	0.19		

IL-1β	PMA	Ct (IL-1β) Mean	ΔCt (IL-1β-β-actin) Mean	S.D.	ΔΔCt	Z'
SuperPrep™	(+)	28.62	7.93	0.15	-5.94	0.74
	(-)	33.99	13.87	0.38		
Company A	(+)	28.00	5.80	0.47	-6.19	0.61
	(-)	33.26	11.99	0.34		

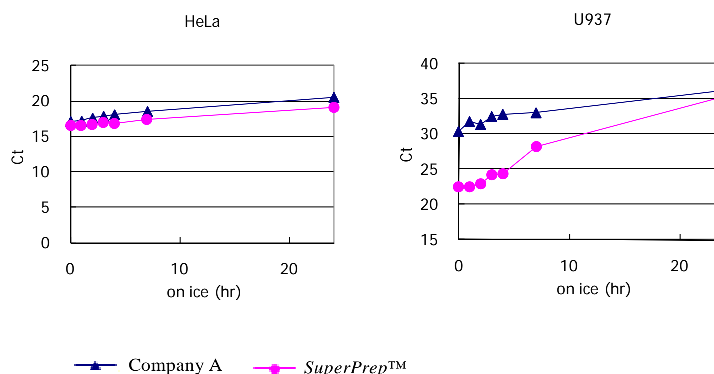
3. Stability test of cell lysates

cDNA were synthesized from lysates that had been left on ice for 0–24 h after lysing of 4×10^4 HeLa and U937 cells using *SuperPrep*™. 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 2 h. Lysates from U937 cells showed higher RNase activity than the other cells and tended to deteriorate in storage over 2 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.



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

[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 2 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 mixing Lysis Solution and Stop 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 **THUNDERBIRD™ SYBR® qPCR Mix**	1.67 mL × 3	QPS-201
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