Table of Contents

I. Description

The CellAmp™ Direct Prep Kit for RT-PCR (Real Time) & Protein Analysis is for preparation of cell lysate from cultured cells that can be directly utilized for both analysis of gene expression by real time RT-PCR, and protein expression by western blot. Using a simple protocol, cell lysate can be prepared within 10 minutes. The prepared cell lysate can be used as template in Takara Bio real time RT-PCR kits, and as a sample for western blots, without the need for additional steps to purify nucleic acids or extract proteins. The included Loading Buffer does not require the addition of 2-mercaptoethanol to perform a western blot.

Lysate preparations can be used with an easy-to-use 1-step real-time RT-PCR kit, such as One Step SYBR® PrimeScript™ RT-PCR Kit II (Perfect Real Time)[1] or One Step SYBR® *Ex Taq* ™ qRT-PCR Kit, and the whole process can be completed in less than two hours. This kit can also be used to prepare template cDNA for real-time PCR in only 30 minutes when combined with a reverse transcription kit such as PrimeScript™ RT reagent Kit (Perfect Real Time)[1] or BluePrint™ RT Reagent Kit for Real Time. The lysate prepared with this kit combines easily with many Takara Bio real time RT-PCR related products.

[1] Product not available in US

II. Kit Components (25 reactions)

(This sample kit contains enough reagent for 25 reactions compared to the regular kit which provides enough for 200 reactions)

(1)	CellAmp™ Washing Buffer	1.63 ml x 2
(2)	CellAmp™ Processing Buffer	1.25 ml
(3)	DNase I for Direct RNA Prep	25 µl
(4)	5 x Loading Buffer	250 µl
(5)	1 M DTT (Dithiothreitol)	50 µl

III. Materials Required but not Provided

1. **Reagents**
   - Real time RT-PCR kit
   - Reagents for SDS-PAGE and Western Blot analysis
2. **Materials**
   - Equipment for SDS-PAGE and Western Blot analysis

IV. Storage −20℃

Note:

- CellAmp™ Washing Buffer and CellAmp™ Processing Buffer can be stored at 4℃ after thawing.
- Store 5 x Loading Buffer at room temperature after opening.
- Avoid contamination.

V. General Considerations

1. This kit may be used in combination with the products listed in Table 1.

Table 1. Compatible real time RT-PCR products.

One-step real time RT-PCR

Cat. #	Product
RR086A/B	One Step SYBR® PrimeScript™ RT-PCR Kit II (Perfect Real Time)[1]
RR066A/B	One Step SYBR® PrimeScript™ RT-PCR Kit (Perfect Real Time)[1]
RR064A/B	One Step PrimeScript™ RT-PCR Kit (Perfect Real Time)[1]
RR067A/B	One Step SYBR® *Ex Taq*™ qRT-PCR Kit
RR068A/B	One Step *Ex Taq*™ qRT-PCR Kit

Two-step real time RT-PCR

Cat. #	Product
RR037A/B	PrimeScript™ RT reagent Kit (Perfect Real Time)[1]
RR091A/B	SYBR® Premix DimerEraser™ (Perfect Real Time)[1]
RR081A/B	SYBR® *Premix Ex Taq*™ II (Perfect Real Time)
RR041A/B	SYBR® *Premix Ex Taq*™ (Perfect Real Time)
RR039A/B	*Premix Ex Taq*™ (Perfect Real Time)
RR737A/B	BluePrint™ RT Reagent Kit (for Real Time)

[1]Product not available in US

2. If precipitate appears during thawing of CellAmp™ Washing Buffer and CellAmp™ Processing Buffer, dissolve precipitate completely before using by warming up to room temperature.
3. Perform the lysate preparation quickly.
4. Be careful to prevent contamination between samples by using new disposable tips for dispensing the reagents. If additional reagent must be withdrawn, change the tip first. Do not use the same tip to pipette different reagents.
5. Guidelines for RNA preparation
   a. Sterilized disposable RNase-free plasticware should be used for these experiments. Any plasticware that is not certified RNase-free should be autoclaved before use. When using glass equipment or spatula, perform dry heat sterilization at 160℃ for at least 2 hours. If dry heat sterilization cannot be performed, treat with 0.1% Diethyl-pyrocarbonate (DEPC) at 37℃ for 12 hours, then autoclave.
   b. It is important to use separate equipment dedicated to RNA experiments.
   c. Reagents should be prepared with 0.1% DEPC treated water as much as possible, and autoclave before use. If reagents are to be used which cannot be autoclaved, then use sterilized equipment and water to prepare the solution, and then perform filter sterilization before use.
   d. Extra precautions should be taken during sample preparation, including use of clean disposable gloves and avoiding RNase contamination from operator sweat or saliva during assembly.

VI. Protocol

1. Preparation of reagents:
(this sample kit includes enough reagent to perform 25 reactions in a 96 well plate)

- Prepare the Processing Solution in a microcentrifuge tube on ice.

Reagents	Per well (using a 96 well plate)
CellAmp™ Processing Buffer	49 µl
DNase I for Direct RNA Prep	1 µl
Total	50 µl

Note: Please refer to Section VII.3 if other types of plates are to be used.

- Add 1M DTT into 5 x Loading Buffer.
  - Aliquot the needed amount of 5 x Loading Buffer into microcentrifuge tubes, and add 10 µl of 1 M DTT for each 100 µl of the 5 x Loading Buffer.

Note: Use 2.5 µl of 5 x Loading Buffer, including DTT, for each 10 µl sample of cell lysate.

2. Preparation of cell lysate from adherent cells cultured on 96 well plates

Note: If a different plate is used, please refer to Section VII.3 for recommended cells/ well and reagent volumes.

a. Dispense cells in a 96 well plate
b. Incubate until cells reach appropriate numbers or confluency.
c. Remove as much culture medium as possible.
d. Add 125 µl of CellAmp™ Washing Buffer to each well and then remove as much Washing Buffer as possible.
e. Add 50 µl of the Processing Solution to each well, then incubate for 5 minutes at room temperature (15 – 28℃).
f. Pipette cell lysate in each well several times and then transfer the lysate to a PCR tube or microcentrifuge tube. Incubate for 5 minutes at 75℃ .
g. Perform real time RT-PCR using the prepared lysate as a template
   - Follow the protocol described in Section VII.1 (one step) or Section VII.2 (two step).
   - For a 25 µl reaction volume of one step real time RT-PCR or 10 µl of reverse transcription, use less than 2 µl of the lysate.
   - The prepared lysate should be kept on ice and real time RT-PCR should start within 1 hour of lysate preparation. The lysate can also be stored for about 2 weeks at −80℃.
h. For western blot, add 2.5 µl 5 x Loading Buffer with DTT into 10 µl of the obtained cell lysate, heat for 5 minutes at 95℃ , and use for SDS-PAGE and western blot.

3. Preparation of cell lysate from 1 x 10^4 - 1 x 10^5 non-adherent cells

Note: If preparing a lysate from > 1 x 10^5 cells, use more reagents (proportionally-you will need to adjust the number of wells used).

a. Count cells and transfer 1 x 10^4 - 1 x 10^5 cells to a microcentrifuge tube.
b. Centrifuge at 300 x g for 5 minutes.
c. Remove as much culture medium as possible.
d. Add 125 µl of CellAmp™ Washing Buffer.

e. Centrifuge at 300 x g for 5 minutes.
f. Remove as much CellAmp™ Washing Buffer as possible.
g. Add 50 µl of the Processing Solution and incubate for 5 minutes at room temperature (15 − 28℃).
h. Pipette cell lysate several times and then transfer the lysate to a PCR tube or microcentrifuge tube. Incubate for 5 minutes at 75℃ .
i. Perform real time RT-PCR using the prepared lysate as a template
   - Follow the protocol described in Section VII.1 (one step) or Section VII.2 (two step).
   - For a 25 µl reaction volume of one step real time RT-PCR or 10 µl of reverse transcription, use less than 2 µl of the lysate.
   - The prepared lysate should be kept on ice and real time RT-PCR should start within 1 hour of lysate preparation. The lysate can also be stored for about 2 weeks at −80℃.
j. For western blot, add 2.5 µl 5 x Loading Buffer with DTT into 10 µl of the obtained cell lysate, heat for 5 minutes at 95℃ , and use for SDS-PAGE and western blot.

VII. Appendix

1. Experimental example of one step real time RT-PCR using One Step SYBR® PrimeScript™ RT-PCR Kit (Perfect Real Time) with Thermal Cycler Dice™ Real Time System

a. Add 1 − 2 µl of cell lysate to a PCR reaction tube or 96 well plate and then place the tube/plate on ice.
b. Prepare master mixture on ice.
   - Volume recommendations are per reaction.
   - The final concentration of primers can be 0.2 µM in most reactions. If the amplification is not satisfactory, then determine the optimal primer concentrations within the range of 0.1 − 1.0 µM.

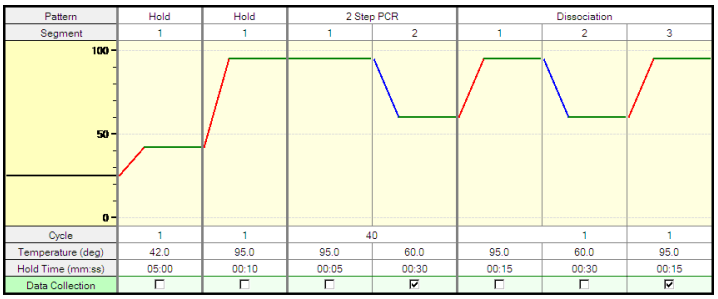
Reagents	Amount	Final concentration
TaKaRa Ex Taq ™ HS (5U / µ l)	0.5 µl	
2 x One Step SYBR® RT-PCR Buffer III	12.5 µl	1 x
PrimeScript™ RT Enzyme Mix II	0.5 µl	
PCR Forward Primer (10 µM)	0.5 µl	0.2 µM
PCR Reverse Primer (10 µM)	0.5 µl	0.2 µM
RNase Free dH_2O	8.5 − 9.5 µl	
Total	23 - 24 µl	

c. Start Reaction

- Add master mix to the cell lysate in PCR tube or 96 well plate and mix well.
- Centrifuge the PCR tube or plate briefly, set on Thermal Cycler Dice™ Real Time System (or other thermal cycler) and start reaction.

Note:

- When using *TaKaRa Ex Taq*™ HS, the initial denaturation step prior to PCR should be at 95℃ for 10 sec. Enzyme activity decreases with longer heat treatment and the amplification efficiency and quantification accuracy can be affected.



Pattern 1 : Reverse Transcription
Hold
42℃ 5 min.
95℃ 10 sec.
Pattern 2 : PCR
Cycle : 40
95℃ 5 sec.
60℃ 30 sec.
Pattern 3 : Dissociation

d. Reaction analysis

- After completing reaction, verify amplification curve and dissociation curve.

2. Experimental example of two step real time RT-PCR using PrimeScript™ RT reagent Kit (Perfect Real Time) and SYBR® *Premix Ex Taq*™ II (Perfect Real Time) with Thermal Cycler Dice™ Real Time System

a. Reverse transcription : PrimeScript™ RT reagent Kit (Perfect Real Time)

1) Prepare the reaction mixture on ice.

- Volume recommendations are per reaction.
- Prepare a slightly larger amount of master mixture than is required for your number of reactions to compensate for pipetting losses. After dispensing aliquots of this mixture into the microtubes, add the RNA sample.

Reagents	Amount	Final concentration
5 x PrimeScript™ Buffer (for Real Time)	2.0 µl	1 x
PrimeScript™ Enzyme Mix I	0.5 µl	
Oligo dT Primer (50 µM)	0.5 µl	25 pmol
Random 6 mers (100 µM)	0.5 µl	50 pmol
RNase Free dH_2O	4.5 – 5.5 µl	
Total	8 – 9 µl	

Note:

- It is possible to scale up the RT reaction as needed.
- BluePrint™ RT Reagent Kit (for Real Time)(Cat. #RR737) may be used for reverse transcription (refer to its protocol for recommended reaction volumes and conditions).
- Efficient synthesis of cDNA from total RNA can be accomplished using both Oligo dT Primer and Random 6 mers.
- If using only Oligo dT Primer, Random 6mers, or Gene specific primer, refer to the amount of primer below.

Primer	Amount	Total Amount
Oligo dT Primer (50 µM)	0.5 µl	25 pmol
Random 6 mers (100 µM)	0.5 µl	50 pmol
Gene specific primer (2 µM)	0.5 µl	1 pmol

2) Add the master mixture prepared above to a reaction tube, and then add 1 − 2 µl of the cell lysate and mix well. Centrifuge the tube briefly, and then incubate it using the following conditions :

   37℃, 15 minutes (Reverse transcription)
   85℃, 5 sec (Inactivation of reverse transcriptase with heat treatment)
   4℃

   Note:

   - Up to 2 µl of the cell lysate can be reverse transcribed in 10 µl of the reaction mixture.
   - When using a gene specific primer, perform reverse transcription at 42℃ for 15 minutes. If non-specific amplification products are observed at the PCR step, resetting this temperature to 50℃ may improve the results.
   - When the reaction mixture obtained in step 2 is used for real time PCR, the volume of the mixture should be less than 10% of the total real time PCR reaction volume.

b. Real time PCR

1) Add 2 µl of the reverse transcription reaction mixture to a PCR reaction tube or 96 well plate and then place on ice.
2) Prepare master mixture on ice.
   - Volume recommendations are per reaction.
   - The final concentration of primers can be 0.4 µM in most reactions. If the amplification is not satisfactory, then determine the optimal primer concentrations within the range of 0.2 − 1.0 µM.

Reagents	Amount	Final concentration
SYBR® *Premix Ex Taq*™ II (2 x)	12.5 µl	1 x
PCR Forward Primer (10 µM)	1.0 µl	0.4 µM
PCR Reverse Primer (10 µM)	1.0 µl	0.4 µM
dH_2O (sterilized distilled water)	8.5 µl	
Total	23 µl	

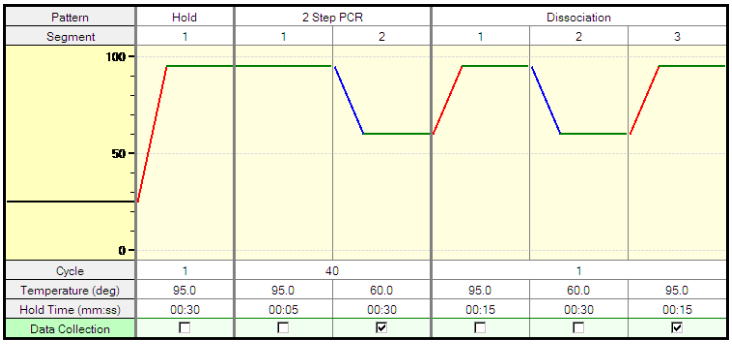
3) Start Reaction

- Add master mix to the cell lysate in PCR tube or 96 well plate and mix well.
- Centrifuge the PCR tube or plate briefly, set on Thermal Cycler Dice™ Real Time System (or other thermal cycler) and start reaction.

Note:

- The recommended PCR conditions (Shuttle PCR standard protocol) are below. Try this protocol first and then change the PCR reaction conditions as needed. 3 Step PCR often works better with primers that have lower Tm values.
- When using SYBR® *Premix Ex Taq*™ II, the initial denaturation step prior to PCR should be at 95℃ for 30 sec. Enzyme activity decreases with longer heat treatments and the amplification efficiency and quantification accuracy can be affected.

[Shuttle PCR standard protocol]



Hold (denaturing)
Cycle : 1
95℃ 30 sec.
2 step PCR
Cycle : 40
95℃ 5 sec.
60℃ 30 sec.
Dissociation

4) Reaction Analysis

- After completing reaction, verify amplification curve and dissociation curve, and create standard curve if quantitative analysis is necessary.

Note: Refer to your Real Time PCR instrument manual for analysis details.

3. Adherent cell number and reagent volume per well of culture plate used (this kit has only enough reagent for 25 wells on a 96 well plate-please adjust proportionally)

	96-well	48-well	24-well	12-well	6-well
Dispensed cells/well	1×10^4 - 1×10^5	2×10^4 - 2×10^5	4×10^4 - 4×10^5	8×10^4 - 8×10^5	2×10^5 - 2×10^6
CellAmp™ Washing Buffer	125 µl	250 µl	500 µl	1 ml	2.5 ml
CellAmp™ Processing Buffer	49 µl	98 µl	196 µl	392 µl	980 µl
DNase I for Direct RNA Prep	1 µl	2 µl	4 µl	8 µl	20 µl

Note: These recommendations are for commonly used adherent cells and culture conditions. The experimental protocol may need to be optimized for your cell number and culture conditions.

4. DNase I (-) protocol (optional)

If DNase I affects the result of the western blot, prepare cell lysates without addition of DNase I for Direct RNA Prep (Please refer to Note at the top of page 15). In this case, design real time primers spanning an exon-exon junction to avoid influence of genomic DNA on real time RT-PCR.

a. Preparation of reagents.

1) Prepare the Solution in a microcentrifuge tube.

Reagents	
5 x Loading Buffer	90 µl
1 M DTT	10 µl
Total	100 µl

Note: Use 2.5 µl of 5 x Loading Buffer, including DTT, for each 10 µl of cell lysate.

b. Preparation of cell lysate from adherent cells cultured on 96 well plates

Note: If a different plate is used, please refer to Section VII. 4. d for recommended cells/well and reagent volumes.

1) Dispense cells in a 96 well plate
2) Incubate until cells reach appropriate numbers or confluency.
3) Remove as much culture medium as possible.
4) Add 125 µl of CellAmp™ Washing Buffer to each well.
5) Remove as much CellAmp™ Washing Buffer as possible.
6) Add 50 µl of CellAmp™ Processing Buffer to each well, then incubate for 5 minutes at room temperature (15 – 28℃) .
7) Pipette cell lysate in each well several times and then transfer the lysate to a PCR tube or microcentrifuge tube. Incubate for 5 minutes at 75℃ .
8) Perform real time RT-PCR using the prepared lysate as a template
   - Follow the protocol described in Section VII.1 (one step) or Section VII.2 (two step).
   - For a 25 µl reaction volume of one step real time RT-PCR or 10 µl of reverse transcription, use less than 2 µl of the lysate.
   - The prepared lysate should be kept on ice and real time RT-PCR should start within 1 hour of lysate preparation. The lysate can be also stored for about 2 weeks at −80℃.
9) For western blot, add 2.5 µl of 5 x Loading Buffer containing DTT into 10 µl of the cell lysate preparation, heat for 5 minutes at 95℃ , and use for SDS-PAGE and western blot.

c. Preparation of cell lysate from 1 x 10^4 - 1 x 10^5 non-adherent cells

Note: If preparing a lysate from > 1 x 10^5 cells, use more reagents (proportionally-this kit has enough reagent for 25 wells).

1) Count cells and transfer less than 1 x 10^5 cells to a microcentrifuge tube.
2) Centrifuge at 300 x g for 5 minutes.
3) Remove as much culture medium as possible.
4) Add 125 µl of CellAmp™ Washing Buffer.
5) Centrifuge at 300 x g for 5 minutes.
6) Remove as much CellAmp™ Washing Buffer as possible.
7) Add 50 µl of CellAmp™ Processing Buffer and incubate for 5 minutes at room temperature (15 − 28℃).
8) Pipette cell lysate in each well several times and then transfer the lysate to a PCR tube or microcentrifuge tube. Incubate for 5 minutes at 75℃ .
9) Perform real time RT-PCR using the prepared lysate as a template
   - Follow the protocol described in Section VII.1 (one step) or Section VII.2 (two step).
   - For a 25 µl reaction volume of one step real time RT-PCR or 10 µl of reverse transcription, use less than 2 µl of the lysate.
   - The prepared lysate should be kept on ice and real time RT-PCR should start within 1 hour of lysate preparation. The lysate can be also stored for about 2 weeks at −80℃.
10) For western blot, add 2.5 µl of 5 x Loading Buffer containing DTT into 10 µl of the cell lysate preparation, heat for 5 minutes at 95℃ , and use for SDS-PAGE and western blot.

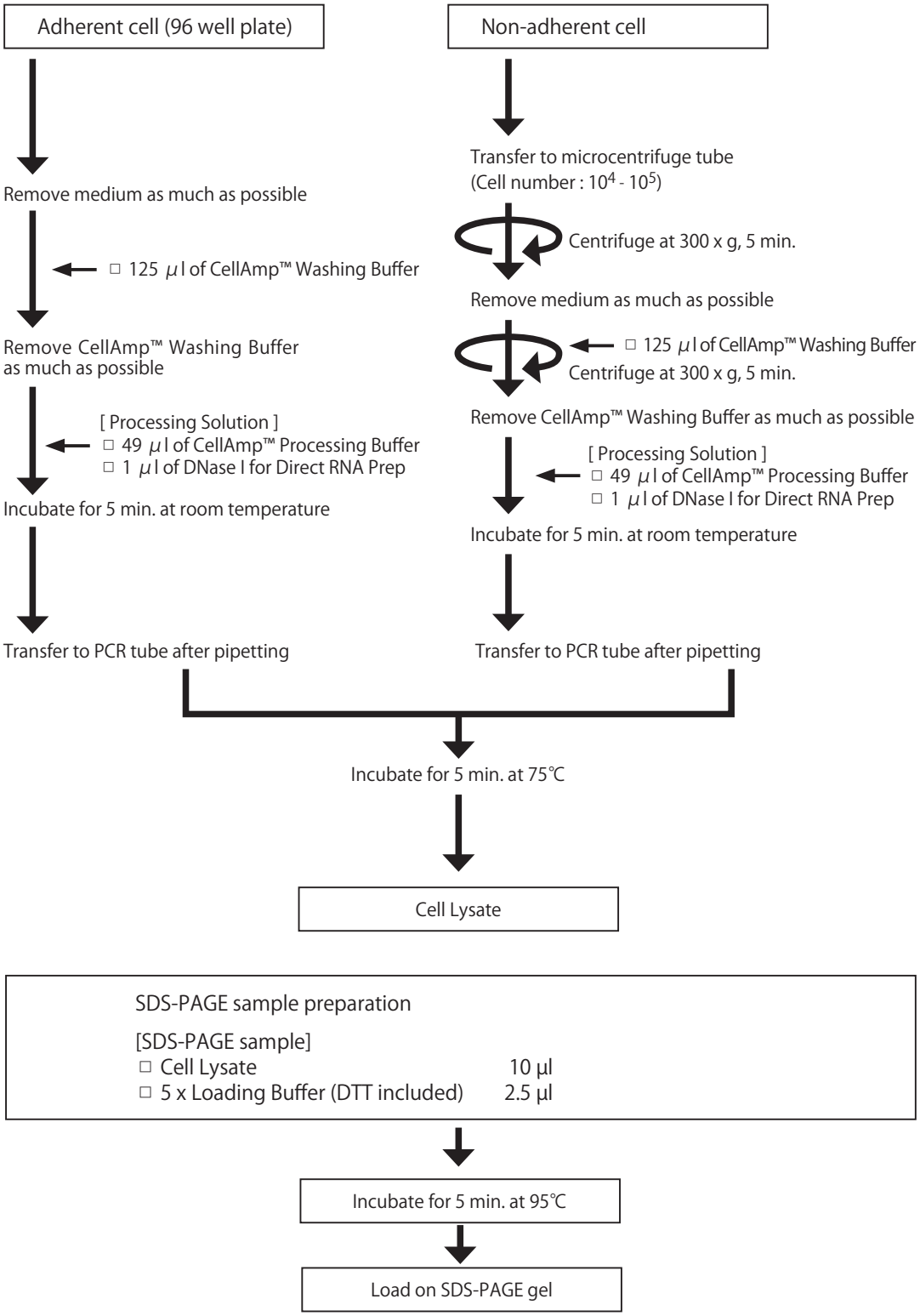
d. Adherent cell number and reagent volume per well of culture plate used (this kit has only enough reagent for 25 wells on a 96 well plate-please adjust)

[DNase I (-) protocol]

	96-well	48-well	24-well	12-well	6-well
Dispensed cells/well	1 x 10^4 - 1 x 10^5	2 x 10^4 - 2 x 10^5	4 x 10^4 - 4 x 10^5	8 x 10^4 - 8 x 10^5	2 x 10^5 - 2 x 10^6
CellAmp™ Washing Buffer	125 µl	250 µl	500 µl	1.0 ml	2.5 ml
CellAmp™ Processing Buffer	50 µl	100 µl	200 µl	400 µl	1,000 µl

Note: These recommendations are for commonly used adherent cells and culture conditions. The experimental protocol may need to be optimized for your cell number and culture conditions.

CellAmp® Direct Prep Kit for RT-PCR (Real Time) & Protein Analysis Work Flow



SDS-PAGE sample preparation

[SDS-PAGE sample]

□ Cell Lysate	10 μl
□ 5 x Loading Buffer (DTT included)	2.5 μl

Incubate for 5 min. at 95℃

Load on SDS-PAGE gel

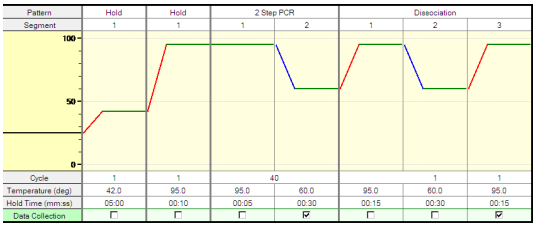
1 step real-time

Add 1 – 2 μl of cell lysate to reaction tube or a well

One Step SYBR® PrimeScript™ RT-PCR Kit (Cat #RR066A/B)
[Master Mix]
< per reaction >

□ RNase Free dH_2O	8.5 – 9.5 μl
□ 2 x One Step SYBR® RT-PCR Buffer III	12.5 μl
□ PCR Forward Primer (10 μM)	0.5 μl
□ PCR Reverse Primer (10 μM)	0.5 μl
□ PrimeScript™ RT Enzyme Mix II	0.5 μl
□ *TaKaRa Ex Taq*™ HS	0.5 μl

Start reaction



Pattern 1 : Reverse transcription
Hold
42℃ 5 min.
95℃ 10 sec.
Pattern 2 : PCR
Cycle : 40
95℃ 5 sec.
60℃ 30 sec.
Pattern 3 : Dissociation

Analyze after reaction is completed.

Note: If One Step SYBR® *Ex Taq*™ qRT-PCR Kit or One Step *Ex Taq*™ qRT-PCR Kit is used, please refer to the product protocol for recommended reagent volumes.

**BluePrint™ RT Reagent for Real Time (Cat. #RR737) can be used

2 step real-time

PrimeScript™ RT reagent Kit (Cat #RR037A/B)**
[Master Mix]
< per reaction >

□ RNase Free dH_2O	4.5 – 5.5 μl
□ 5 x PrimeScript™ Buffer (for Real Time)	2.0 μl
□ Oligo dT Primer (50 μM)	0.5 μl
□ Random 6 mers (100 μM)	0.5 μl
□ PrimeScript™ RT Enzyme Mix I	0.5 μl

Add 1 – 2 μl of cell lysate to reaction tube or a well

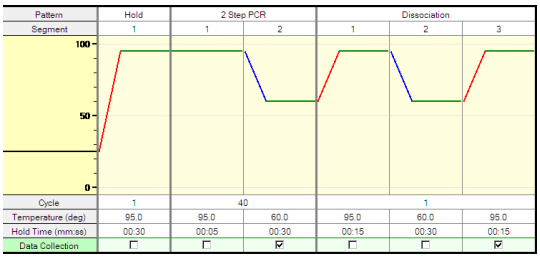
37℃ 15 min. (reverse transcription)
85℃ 5 sec. (Inactivation)
4℃

Add 2 μl of reaction mixture of reverse transcription to a tube or a well.

SYBR® *Premix Ex Taq*™ II (Cat #RR081A/B)
[Master Mix]
< per reaction >

□ RNase Free dH_2O	8.5 μl
□ PCR Forward Primer (10 μM)	1.0 μl
□ PCR Reverse Primer (10 μM)	1.0 μl
□ SYBR® *Premix Ex Taq*™ II (2 x)	12.5 μl

Start reaction



Pattern 1 : (Initial denaturing)
Hold
95℃ 30 sec.

Pattern 2 : PCR
Cycle : 40
95℃ 5 sec.
60℃ 30 sec.
Pattern 3 : Dissociation

Analyze after reaction is completed.

VIII. Experimental Example

1. Time-dependent change of both mRNA and protein expressiom of heme oxygenase decycling 1 (Hmox1) by drug-stimulation

Methods :

1. RAW 264.7 cells were dispensed in a 24 well plate (4 x 10^5 cells per well), cultured for about 16 hours, and treated with an Hmox1 inducing drug. Cell lysates (200 µl each) were prepared using this kit at 1, 2, 4, 6 and 8 hours after adding the drug. Control lysate samples were also prepared without the addition of the inducing drug.
2. Analysis of Hmox1 mRNA expression by real-time RT-PCR.
   - 2 µl of undiluted cell lysate, and 2 µl of lysate diluted 10-fold with CellAmp® Processing Buffer were used as templates for analysis of Hmox1 mRNA expression by real time RT-PCR using One Step SYBR® PrimeScript® RT-PCR kit (Perfect Real Time). Gene expression of the reference gene, Gapdh, was used as a control.
3. Analysis of Hmox1 protein expression by western blot
   - 2.5 µl of 5 x Loading Buffer(with DTT) was added into 10 µl of undiluted cell lysate. SDS-PAGE was conducted after heating the samples at 95℃ for 5 minutes. Hmox1 protein was probed with Anti-Heme Oxygenase-1 (GST-1), Monoclonal, POD (Cat. #M177), and chemiluminescent SuperSignal West Femto Maximum Sensitivity Substrate (ThermoFisher Scientific, Cat. #34095). Signals were detected by a luminometer, LuminoShot 400Jr, with an exposure time of 1 minute.

Results :

It was confirmed that the C_t values of Hmox1 decreased and the expression level of Hmox1 mRNA increased in a time-dependant manner after stimulation as shown in the results of real time RT-PCR using both undiluted and diluted cell lysates (Figure 1). In addition, it was also confirmed that the expression level of Hmox1 protein increased corresponding to its mRNA expression level as higher signal intensity of Hmox 1 protein was detected.

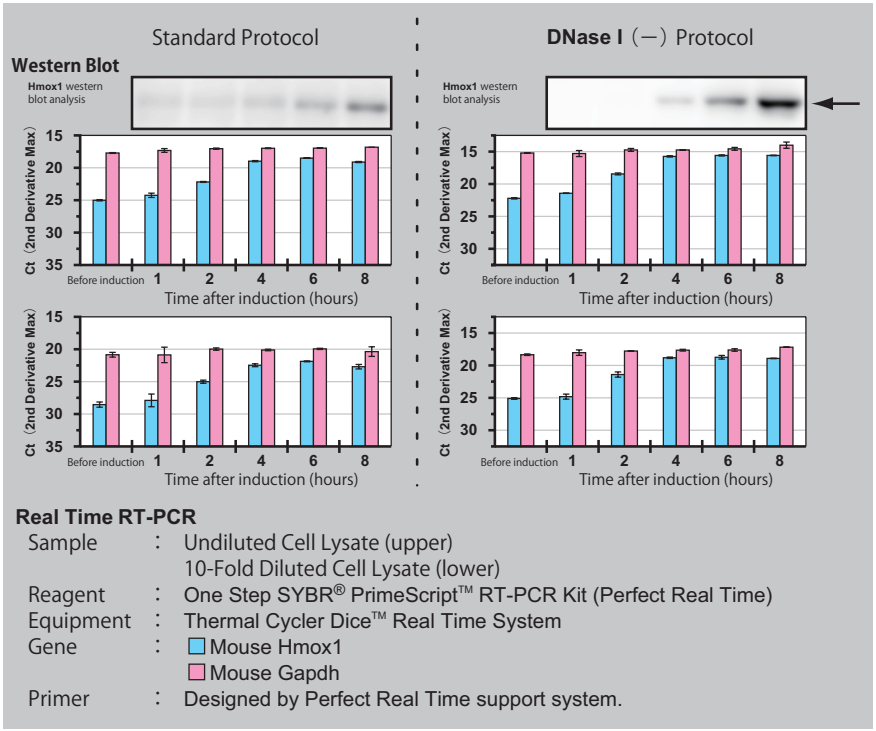


Figure 1. Comparison between western blot and real time RT-PCR results.

IX. Troubleshooting

1. No amplification with real time RT-PCR

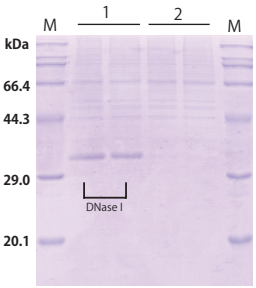
- Reconsider PCR primer design. Refer to the One Step SYBR® PrimeScript™ RT-PCR Kit (Perfect Real Time) (Cat. #RR066A/B) or One Step SYBR® PrimeScript™ RT-PCR Kit II (Perfect Real Time) (Cat. #RR086A/B) protocol.
- Depending on cell species or culture conditions, the number of cells or experimental protocol may need to be optimized.
- Wash cells with CellAmp™ Washing Buffer and remove contaminants in the culture medium. Remove as much of the culture medium and CellAmp™ Washing Buffer as possible.
- Prepare real time RT-PCR reaction mixture on ice and protect from light until starting the reaction.
- When too much cell lysate volume is used in reverse transcription or one step RT-PCR, reaction efficiency might be reduced. Reduce the cell lysate volume.
- Do not mix primers and cell lysate directly. Primers might be digested by residual DNase activity in the cell lysate.

2. Signals cannot be detected on western blot

- Signals cannot be detected by analysis on western blots because the amount of cells used is too small. In this case, adjust the experimental protocol to use more cells than are listed in Section VII.4.d. However, this might inhibit RT-PCR. In that case, dilute the cell lysate for real time RT-PCR using CellAmp™ Processing Buffer.

Note:

This kit can effectively remove genomic DNA using DNase I for Direct RNA Prep. Therefore, it is a very powerful tool for analyzing gene expression using real time RT-PCR and overcomes the problems associated with contaminating genomic DNA (e.g., the primer cannot be designed to span to an exon-exon junction complex, or analyzing genes with low expression). In the standard protocol, because DNase I for Direct RNA Prep is added when preparing cell lysate, a 31kDa band of DNase I will appear on SDS-PAGE gels as shown in Figure 2. If you find that DNase I for Direct RNA Prep has influenced the result of the western blot, please prepare cell lysate using the DNase I (-) protocol (Section VII.4). In addition, design primers to span an exon-exon junction to prevent genomic DNA from influencing the results of the real time RT-PCR.



M：Protein Molecular Weight Marker (Broad) (Cat. #3452)
Sample：HeLa cell lysate 10 µl
(8 x 10^4 cells/well, prepared from a 24-well plate)

1：Standard Protocol
2：DNase I (-) Protocol

Figure 2. SDS-PAGE of Cell Lysate (CBB Stained).

X. Related Products

One Step SYBR® PrimeScript™ RT-PCR Kit II (Perfect Real Time) (Cat. #RR086A/B) ＊1
One Step SYBR® PrimeScript™ RT-PCR Kit (Perfect Real Time) (Cat. #RR066A/B) ＊1
One Step PrimeScript™ RT-PCR Kit (Perfect Real Time) (Cat. #RR064A/B) ＊1
PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B) ＊1
BluePrint™ RT Reagent Kit (for Real Time) (Cat. #RR737A/B)
One Step SYBR® *Ex Taq*™ qRT-PCR Kit (Cat. #RR067A/B)
One Step *Ex Taq*™ qRT-PCR Kit (Cat. #RR068A/B)
SYBR® Premix DimerEraser (Perfect Real Time) (Cat. #RR091A/B)
SYBR® *Premix Ex Taq*™ II (Perfect Real Time) (Cat. #RR081A/B)
SYBR® *Premix Ex Taq*™ (Perfect Real Time) (Cat. #RR041A/B)
Premix Ex Taq™ (Perfect Real Time) (Cat. #RR039A/B)
Thermal Cycler Dice™ Real Time System (Cat. #TP800) ＊2

＊1 : This product is not available in the U.S.

＊2 : This instrument is not available in the U.S. and Europe.