Cat. # 6142

For Research Use

TaKaRa

Synthetic siRNA Quantitation Core Kit

Product Manual

v202203Da

Table of Contents

I. Description

Synthetic siRNA Quantitation Core Kit is designed to quantify synthetic siRNA when used in combination with realtime PCR analysis, and is used to assay the residual amount of the administered siRNA in total RNA samples extracted from cells, tissues, blood, and other specimens.

Small interfering RNA (siRNA), sometimes also referred to as silencing RNA or short interfering RNA, is double stranded RNA, typically 20 - 25 nucleotides in length. siRNA can interfere with specific gene expression in cells by hybridizing to the corresponding mRNA, resulting in mRNA degradation.

Synthetic siRNA is used as a tool to understand protein function. An siRNA is designed that complements a target gene's mRNA sequence. It is introduced into a cell, where it will hybridize to the target mRNA, resulting in mRNA degradation. Without mRNA, target protein expression is knocked out and its function can be assessed by studying the effect of the protein's removal from the cell. Synthetic siRNA has been tested successfully in several disease models in animals. Synthetic siRNA quantitation in cells, tissues and blood plays an important role in siRNA drug development.

Synthetic siRNA generally have two deoxythymidine nucleotides d(TT) as an overhang at the 3′ ends. The Synthetic siRNA Quantitation Core Kit adds polydeoxyadenine (poly dA) to the 3′ overhangs and then carries out a reverse transcription reaction with a specific oligo dT primer, allowing preparation of complementary DNA from synthetic siRNA molecules. High-sensitivity quantitation of the synthetic siRNA can be achieved by using the prepared cDNA as template in an intercalator-based realtime PCR with TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B) or a similar product.

Note:

- This kit is designed to quantify synthetic siRNA with deoxythymidines d(TT) as a 3′ overhang.
- For synthetic siRNA with 3′ overhangs other than d(TT), prepare a specific oligo dT primer with a modified sequence.
- This product does not work with synthetic siRNAs that don't have 3′ overhangs or with RNA 3′ overhangs.

II. Components (for 30 reactions)*1

1.	Terminal Transferase Buffer	390 µl
2.	Terminal Transferase	30 µl
3.	dATP	150 µl
4.	Recombinant RNase Inhibitor	30 µl
5.	Oligo dT Primer*2	150 µl
6.	RT Buffer	270 µl
7.	RT Enzyme Mix	30 µl
8.	Universal Primer (10 µM)	120 µl
9.	EASY Dilution (for Realtime PCR)	1 ml
10.	Control siRNA (20 nM)	10 µl
11.	Control Primer (10 µM)	20 µl

*1 Contains enough reagents for 30 reactions of poly dA tailing and reverse transcription and enough Universal Primer for 120 realtime PCR reactions.

*2 Specially designed Oligo dT Primer for this product.

III. Materials Required but not Provided

1. Reagent

- siRNA sequence-specific primer for realtime PCR (refer to Appendix-2)
- Realtime PCR reagents

  Realtime PCR-related products compatible with this product
  - TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/B)
  - TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/B)
- RNA extraction kit [e.g., RNAiso Plus (Cat. #9108/9109)]

2. Material

- Realtime PCR instrument and compatible microtubes and plates
- 0.2 ml microtubes (PCR tubes)
- Micropipettes and tips (autoclaved)

IV. Storage -20℃

V. Principle

1. Prepare cDNA from synthetic siRNA

< Poly dA tailing >

Heat denature synthetic siRNA to yield a single-stranded sample then add poly dA to d(TT) at 3′ ends of the synthetic siRNA.

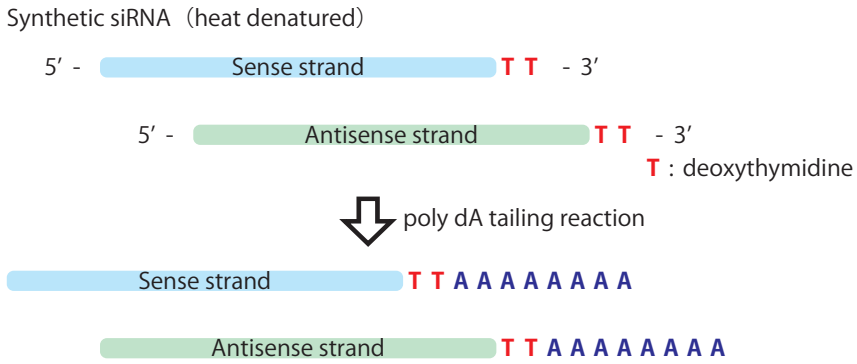


Figure 1. Poly dA tailing.

< Reverse transcription >

A reverse transcription reaction follows the annealing of the Oligo dT Primer to the added poly dA sequence. This Oligo dT Primer has a universal sequence that is used in subsequent realtime PCR.

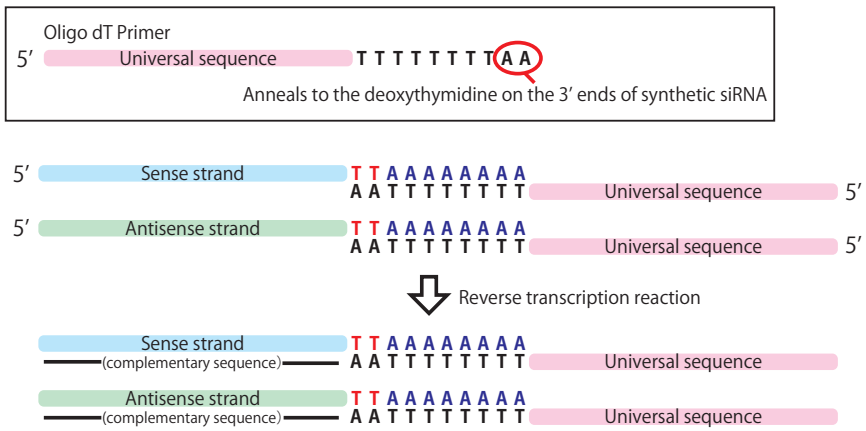


Figure 2. Reverse transcription reaction.

Note: The Oligo dT Primer supplied in this kit is designed for synthetic siRNA with d(TT) in the 3′ overhangs. For siRNA with 3′ overhangs other than d(TT), an oligo dT primer with a sequence corresponding to the 3′ overhang is required. Please contact Takara Bio for inquiries regarding sequences.

2. Quantification by realtime PCR

A realtime PCR with the prepared cDNA as template is carried out using TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/B).*1 Amplification is achieved with the Universal Primer*2 and an siRNA sequence-specific primer.*1 TB Green *Premix Ex Taq* II (Tli RNaseH Plus) uses *TaKaRa Ex Taq*® HS as a hot-start PCR enzyme, thus reducing nonspecific amplification and allowing high-sensitivity quantitative PCR.

*1 To be prepared separately.
*2 Supplied in this product.

< Principle of quantitative by realtime PCR >

In realtime PCR, quantitative analysis is based on the Ct value (Ct: threshold cycle). Threshold fluorescence is the value at which the amount of product reaches a detectable level (i.e. above background) and the Ct value is the number of cycles required for any one reaction to reach the threshold. The correlation between the Ct value and the log of amount of input template is linear.

A standard curve shown in Figure 3 can be plotted based on reaction results using serially diluted standard templates. Determining the Ct value of an unknown sample in the same manner will allow calculation of the amount of input template by plugging that value into this standard curve. Software installed on a realtime PCR device will automatically perform this series of analysis.

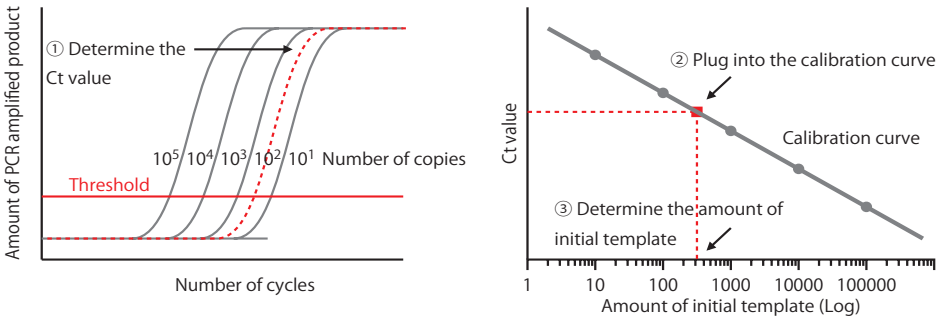


Figure 3. Quantitative analysis of a sample with unknown amount of initial template.

VI. Precautions for Use

This section describes precautions for using this product. Please read through the following section before starting the protocol.

1. General Precaution

- Preparation of a master mix of reagents, including buffers, enzymes, etc. prior to assembling the reaction is recommended. Use of this mixture allows for accurate dispensing of reagents, minimizing experimental variability.
- Gently spin down the terminal transferase, recombinant RNase inhibitor and RT enzyme mix prior to pipetting. Pipet enzymes slowly and carefully because of the viscosity of the 50% glycerol in these solutions.
- Use new disposable pipette tips to avoid contamination between samples when transferring reagents.
- To achieve better quantitative results, warm up the thermal cycler to the desired temperature before starting incubation.

2. Precautions for quantitative realtime PCR

A) Standard curve

The standard curve is based on realtime PCR of serially diluted standard templates. It is not only used to quantify unknown samples, but also to gain useful information such as PCR amplification efficiencies and quantifiable ranges. The key characteristics of a standard curve are its slope and linearity.

- The slope allows determination of PCR amplification efficiency; 80 - 120% is considered appropriate.
- The linearity should have a correlation coefficient (R^2) of 0.98 or above. If low-concentration or high-concentration points deviate from the linear line, remove such points to maintain a correlation coefficient of 0.98 or above.
- Software installed on the realtime PCR device will automatically analyze PCR amplification efficiency and the correlation coefficient (R^2).

B) Standard-sample dilution

Dilute the standard siRNA appropriately to prepare 5 - 7 serial dilutions. At least five steps of serial dilution are required to plot a reliable standard curve for the quantitation of a target siRNA.

[Example: standard-sample dilution]

- 6 steps of 1 : 10 dilution
  Example : 2 nM, 200 pM, 20 pM, 2 pM, 200 fM, 20 fM
- 6 steps of 1 : 5 dilution
  Example : 2 nM, 400 pM, 80 pM, 16 pM, 3.2 pM, 640 fM

It is important to accurately dilute a synthetic siRNA sample down to low concentrations to plot appropriate standard curves. EASY Dilution (for Realtime PCR), which is supplied in this product, is recommended for dilution. Using water or TE buffer for dilution results in unstable dilutions, particularly at low concentrations, thus narrowing the usable range of standard curves.

C) Verify reactivity of siRNA-specific primer

Before using a new primer to quantitate synthetic siRNA in blood, cells or other tissue, verify its reactivity using the following steps.

- Prepare serial dilutions from the target synthetic siRNA. Then use this product to create cDNAs and carry out realtime PCR to verify the PCR amplification efficiency, presence/absence of any nonspecific amplified products and the quantifiable range. A proper range is between 70 and 120% for the amplification efficiency determined here, which is subject to influence by poly dA tailing reaction and reverse transcription reaction.
- Use this product to create cDNA from the target synthetic siRNA. Prepare serial dilutions of the cDNA and then carry out realtime PCR to verify the PCR amplification efficiency, presence/absence of any nonspecific amplifications and the quantifiable range.
- When verifying reactivities, be sure to run a no-template control (NTC) reaction concurrently to check for the presence of any primer-dimers and nonspecific amplified products.
- Use this product to create cDNA from total RNA that does not contain synthetic siRNA and then carry out realtime PCR to make sure there are no amplifications from total RNA. In the event any amplification was detected, verify by melting curve analysis to see if this amplified product can be differentiated from the product amplified from cDNA prepared with the synthetic siRNA.

3. General precautions for handling RNA

- Commercially available sterile disposable plastic equipment are generally RNase-free and should be used, but microtubes for centrifugation and micropipette tips should be autoclaved before use.
- Use dry heat sterilization (160℃ for 2 hours or longer) on all glass equipment, spatulas, etc. For equipment that cannot be sterilized by this method, treat before use in 0.1% diethylpyrocarbonate (DEPC) at 37℃ for 12 hours followed by autoclave treatment (to prevent carboxymethylation of RNA by DEPC).
- It is recommended that all the equipment be used exclusively for RNA experiments.
- The greatest source of RNase contamination is introduced by the operator. When conducting RNA experiments, wear disposable plastic gloves and a mask.

VII. Protocol

1. RNA extraction/purification

Use an RNA extraction kit such as RNAiso Plus (Cat. #9108/9109)*1 to extract and purify total RNA from cells, tissues, blood or any other specimen that contains a synthetic siRNA.

*1 Use RNA-preparation reagents capable of purifying small RNAs.

2. Heat denaturation

A) Prepare the following reaction mixture on ice.
Dispense 5 µl aliquots of dATP into individual reaction tubes and add 5 µl of a synthetic siRNA sample (e.g., extracted and purified total RNA*2 or a diluted sample of synthetic siRNA) to each.

Reagent	Amount
dATP	5 µl
Synthetic siRNA sample	5 µl
Total	10 µl

*2 Use no more than 200 ng of total RNA.

B) Heat denature by incubating the mixture at 75℃ for 5 minutes*3, then quench it on ice. Carry out poly dA tailing reaction immediately after heat denaturation.*4

*3 The temperature for heat treatment differs depending on the siRNA sequence. If an unsatisfactory quantitative result was obtained, try another temperature between 70 and 80℃.

*4 To prevent re-annealing of heat-denatured siRNA, do not leave samples on ice for more than 15 minutes before carrying out the subsequent poly dA tailing reaction.

3. Poly dA tailing reaction

A) Prepare the following reaction mixture on ice.
Prepare slightly more master mix than is required and then add 15 µl of the master mix to each 10 µl aliquot of heat-denatured sample. Be careful to avoid contamination between samples.

Reagent	Amount	
Terminal Transferase Buffer	13 µl	Master mix
Recombinant RNase Inhibitor	1 µl	
Terminal Transferase	1 µl	
heat-denatured sample	10 µl	
Total	25 µl	

B) Gently agitate the reaction mixture and allow the poly dA tailing reaction to take place.

1) 37℃ 30 min
2) 4℃

4. Oligo dT primer annealing

A) Prepare the following reaction mixture on ice.
Dispense 5 μl aliquots of Oligo dT Primer into individual reaction tubes and then add 5 μl of the poly dA tailing reaction mixture to each tube.

Reagent	Amount
Oligo dT Primer	5 μl
Poly dA tailing reaction mixture	5 μl
Total	10 μl

B) Incubate at 75℃ for 5 minutes and then quench on ice. After the annealing reaction, immediately carry out the reverse transcription reaction.

5. Reverse transcription reaction

A) Prepare the following reaction mixture on ice.
Prepare slightly more master mix than is required and then add 10 μl of the master mix to each aliquot of the annealing reaction mixture. Be careful to avoid contamination between samples.

Reagent	Amount	
RT Buffer	9 μl	Master mix
RT Enzyme Mix	1 μl	
Annealing reaction mixture	10 μl	
Total	20 μl	

B) Gently agitate the reaction mixture and allow the reverse transcription reaction to take place.

1) 42℃ 15 min (reverse transcription)
2) 85℃ 5 sec (heat-inactivation of reverse transcriptase)
3) 4℃

6. Perform realtime PCR (Refer to Section VIII)

VIII. Appendix

1. Realtime PCR reaction using TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/B) (For Thermal Cycler Dice™ Real Time System *II* (discontinued))

A) Prepare the following reaction mixture on ice.

< Per reaction >

Reagent	Amount	Final conc.
TB Green *Premix Ex Taq* II (2X)	12.5 μl	1X
Universal Primer (10 μM)	1.0 μl	0.4 μM ＊1
siRNA qPCR Primer ＊2 (10 μM)	1.0 μl	0.4 μM ＊1
Reverse transcription reaction mixture	2.0 μl ＊3	
Sterile purified water	8.5 μl	
Total	25 μl ＊4	

＊1 The final concentration of primers can be 0.4 μM in most reactions. When it does not work, determine the optimal primer concentration within the range of 0.2 - 1.0 M

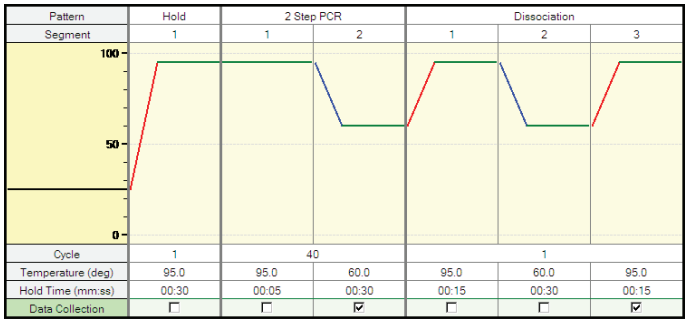
＊2 Prepare a primer specific to the synthetic siRNA being quantified. Refer to Section VIII. 2 for guidelines on primer design. Use the control primer when using cDNA prepared from the control siRNA.

＊3 The reverse transcription reaction mixture should be added at less than 10% of the PCR reaction mixture volume.

＊4 The recommended reaction mixture volume is 25 μl.

B) Mix the above reaction mixture well. Spin down the reaction tubes or plates and place them into the Thermal Cycler Dice Real Time System *II* (discontinued)(or other realtime PCR instrument) to begin the reaction.

Note: The shuttle PCR standard protocol (below) is recommended. Try this protocol first, and then optimize reaction conditions if needed. Before optimization, be sure to read the section on how to select reaction conditions provided in the manual for TB Green *Premix Ex Taq* II.



Pattern 1 : Initial denaturation
Hold
95℃ 30 sec
Pattern 2 : PCR reaction
Cycles : 40
95℃ 5 sec
60℃ 30 - 60 sec
Pattern 3 : Dissociation

Figure 4. Shuttle PCR standard protocol.

[attention]
TB Green *Premix Ex Taq* II uses an enzyme for hot start PCR utilizing *Taq* antibody. The initial denaturation step prior to PCR should be at 95℃ for 30 sec. Longer heat treatment (e.g., 95℃ for 5 - 15 min), may result in lower enzyme activity, and both amplification efficiency and quantification accuracy can be negatively affected.

C) After the reaction is complete, verify the amplification and melting curves and plot a standard curve if a quantitative determination is done.

For analytical method, refer to the manual for Thermal Cycler Dice Real Time System *II* (discontinued) or your realtime PCR instrument.

2. How to design siRNA sequence-specific primers for realtime PCR

A) Using the siRNA sequences, excluding the 3′ overhangs, replace uracils (U) with thymines (T) (see figure below).

B) Design a specific primer for the antisense strand or the sense strand and use it to carry out realtime PCR in advance to verify the standard curve. Make sure there are no nonspecific amplifications and that the quantifiable range is adequate.

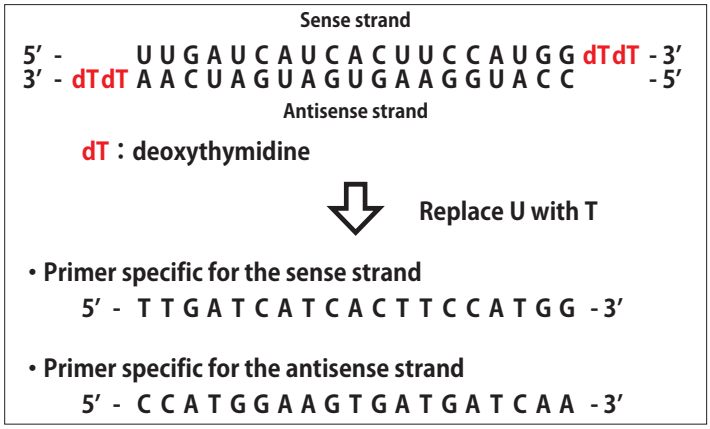


Figure 5. siRNA sequence-specific primer design. (For realtime PCR)

3. Preparing a standard curve for control siRNA

EASY Dilution (for Realtime) was used to prepare 10 serial dilutions of the control siRNA with concentrations from 2 nM to 20 fM and cDNA was prepared subsequently in accordance with the protocol for this product. Realtime PCR was then carried out with the cDNA as the template using TB Green *Premix Ex Taq* II (Tli RNaseH Plus), and a standard curve was plotted based on the results.

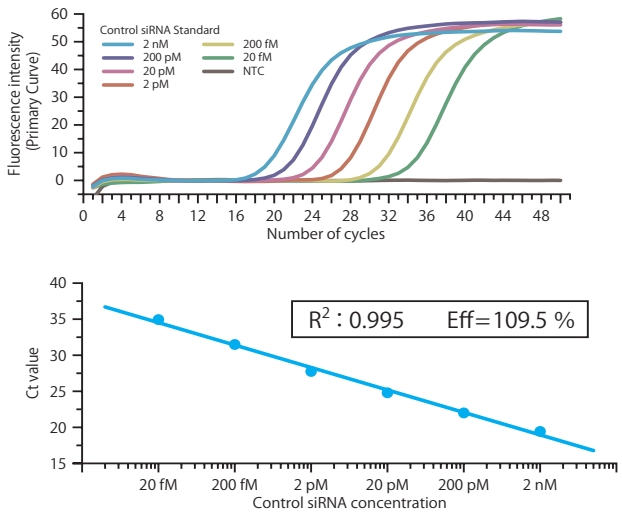


Figure 6. Standard curve for control siRNA.

4. Procedures for quantifying synthetic siRNA using Synthetic siRNA Quantitation Core Kit

Dispense 5 μl aliquots of dATP into individual reaction tubes and add to each tube 5 μl of a synthetic siRNA sample (e.g., total RNA or diluted synthetic siRNA sample)

↓

75℃ 5 min (heat denaturation)

↓

Quench on ice

↓

[Master Mix]
< Per reaction >

☐ Terminal Transferase Buffer	13 μl
☐ Recombinant RNase Inhibitor	1 μl
☐ Terminal Transferase	1 μl

↓

For each reaction, add 15 μl of the master mix to each heat-denatured sample. (Take care to avoid contamination between samples.)

↓

37℃ 30 min (poly dA tailing reaction)
4℃

↓

Dispense 5 μl aliquots of the oligo dT primer to individual reaction tubes and add 5 μl of the poly dA tailing reaction mixture to each.

↓

75℃ 5 min (Oligo dT primer annealing)

↓

[Master Mix]
< Per reaction >

☐ RT Buffer	9 μl
☐ RT Enzyme Mix	1 μl

↓

For each reaction, add 10 μl of the master mix to each annealing reaction mixture. (Take care to avoid contamination between samples.)

↓

42℃ 15 min (reverse transcription)
85℃ 5 sec (heat inactivation of reverse transcriptase)
4℃

↓

Template DNA

↓

Realtime PCR
(With Thermal Cycler Dice Real Time System *II*: discontinued)

↓

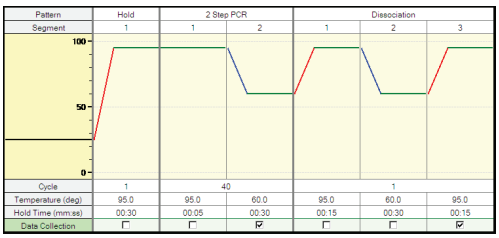
TB Green *Premix Ex Taq* II (Tli RNaseH Plus)
[Master Mix]
< Per reaction >

☐ Sterile purified water	8.5 μl
☐ Universal Primer (10 μM)	1 μl
☐ siRNA qPCR Primer (10 μM)	1 μl
☐ TB Green *Premix Ex Taq* II (2X)	12.5 μl

↓

Dispense 23 μl aliquots of the master mix into individual reaction tubes and add 2 μl of the reverse transcription reaction mixture to each.

↓



Initiate reaction.

Pattern 1 : (Initialdenaturation)
Hold
95℃ 30 sec
Pattern 2 : PCR reaction
Cycles : 40
95℃ 5 sec
60℃ 30 - 60 sec
Pattern 3 : Dissociation

↓

After the reaction is complete, analyze data

IX. Experimental Examples

1. Model experiment for quantifying synthetic siRNA in mouse blood samples

- 10 amol, 1 fmol and 100 fmol of control siRNA were added to samples of mouse whole blood in 50 μl aliquots (anticoagulant: sodium citrate).
- Then total RNA was prepared using RNAiso Plus (Cat. #9108) and Dr. GenTLE™ Precipitation Carrier (Cat. #9094) (pellet dissolved in 50 μl).
- This product and TB Green *Premix Ex Taq* II (Perfect Real Time) (Cat. #RR081A/B; discontinued) were used to carry out realtime PCR to determine the quantities of control siRNA.
- The current, we recommend the use of RR820A/B in the version up of RR081A/B.

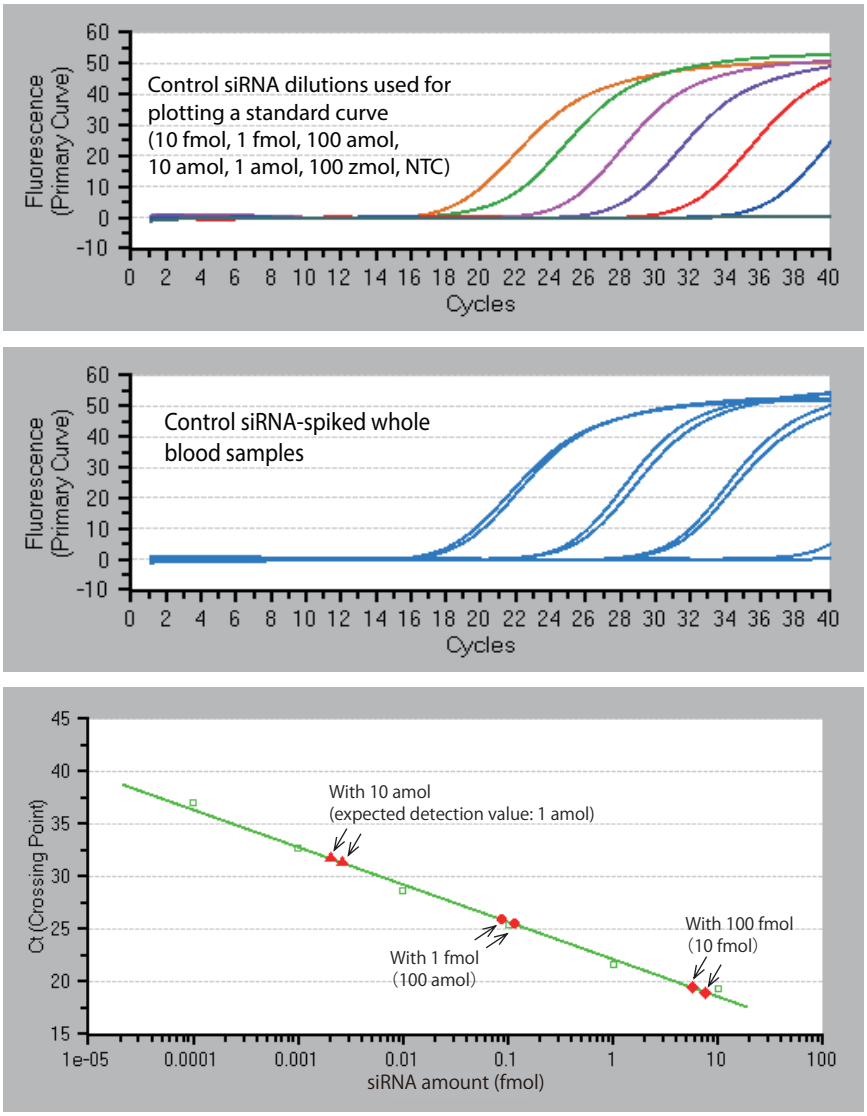


Figure 7. Quantitation of control siRNA spiked into mouse blood samples.

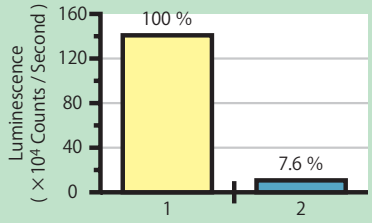
Result : siRNA quantities determined using this product followed by realtime PCR correlated with the amount of siRNA added to mouse blood samples.

2. Quantification of synthetic siRNA transfected into cultured cells

Cultured cells were transfected with synthetic siRNA, and the RNAi effect was subsequently confirmed. Total RNA was prepared from the transfected cultured cells, and assayed for the residual amount of siRNA using this product.

- HEK293 cells were seeded into a 24-well plate at 1 x 10^5 cells/well and incubated for about 24 hours. Then co-transfection was carried out: secreted luciferase (MetLuc2) expression vector pMetLuc2-Control (500 ng) by *Trans*IT-LT1 (Mirus Bio) and MetLuc2 siRNA (7.5 pmol), which knockdowns MetLuc2 gene expression, by *Trans*IT-TKO (Mirus Bio) (carried out in 2 wells).
- After 24 hours, culture supernatants were harvested for luciferase luminescence assay, and 50 µl of RNA sample was prepared from each.*
- Subsequently, EASY Dilution (for Real Time) was used to make 100-fold dilutions, from which 5 µl aliquots were taken to serve as assay samples and cDNAs were generated according to this product's protocol. Realtime PCR was then carried out with the cDNA as the template using TB Green *Premix Ex Taq* II (Perfect Real Time) and an antisense-strand-specific primer to determine the amounts of MetLuc2 siRNA.

  * To determine the siRNA recovery rate in RNA extractions, homogenized cells were spiked with a known amount of the internal standard siRNA (1 pmol).



		Luminescence	Relative Luminescence
1	Control	141×10^4	100 %
2	MetLuc2 siRNA	11×10^4	7.6 %

Figure 8. Confirmation of knockdown effect.

A relative luminescense of cells transfected with MetLuc2 siRNA to the control siRNA showed a mean of 7.6 % (Well 1: 7.8% and Well 2: 7.4%), demonstrating a knockdown effect by RNAi.

	Known amount	MetLuc2 siRNA		Internal standard siRNA	
		Ct	Assay result	Ct	Assay result
Standard 1	10 fmol	20.71	–	17.38	–
Standard 2	1 fmol	24.53	–	20.89	–
Standard 3	100 amol	28.85	–	24.61	–
Standard 4	10 amol	32.73	–	28.55	–
Standard 5	1 amol	36.43	–	32.17	–
Sample (Well 1)	–	28.73	96 amol	21.79	610 amol
Sample (Well 2)	–	27.47	199 amol	21.67	657 amol

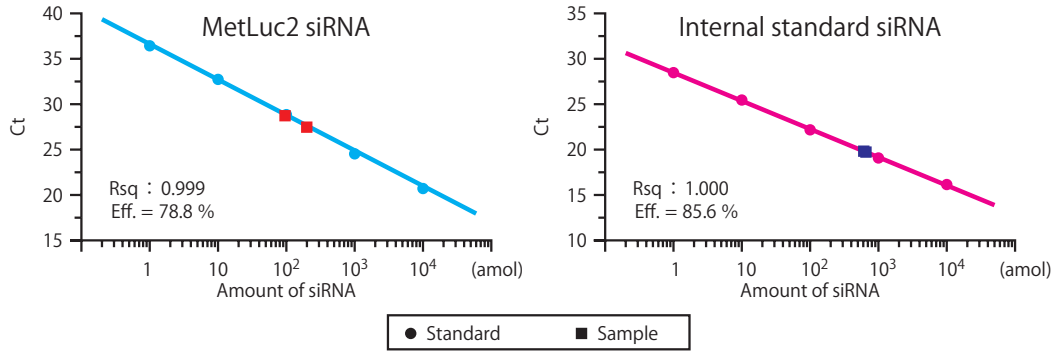


Figure 9. Quantification of synthetic siRNA transfected into cultured cells.

Standard curves were respectively plotted using 10-fold serial dilutions of the internal standard siRNA and MetLuc2 siRNA. Assay results for the internal standard siRNA and MetLuc2 are shown.
The siRNA recovery rate was determined by the equation below based on the quantities determined for the internal standard siRNA that was spiked into the RNA preparation.

Sample (Well 1):

$$\text{siRNA recovery rate} = \frac{610\ \text{amol} \times 100 \times 50}{5 \times 1\ \text{pmol}} \times 100 = 61.0\ (\%)$$

Amount of internal standard siRNA determined by realtime PCR
Dilution ratio of RNA sample
Amount of RNA prepared from cells
Amount of RNA sample used with this product
siRNA recovery rate

The residual amount of MetLuc2 siRNA in cultured cells was determined based on the amount of MetLuc2 siRNA determined from realtime PCR and the siRNA recovery rate (above).

Sample (Well 1):

$$\text{MetLuc2 siRNA quantification result} = \frac{96\ \text{amol} \times 100 \times 50}{5 \times 61.0/100} = 157\ \text{fmol}$$

Amount of internal standard siRNA determined by realtime PCR
Dilution ratio of RNA sample
Amount of RNA prepared from cells
Amount of RNA sample used with this product
siRNA recovery rate

These results showed that the amounts of residual MetLuc2 siRNA were 157 fmol in the sample from Well 1 and 303 fmol in the sample from Well 2.

X. Troubleshooting

1. No amplification seen in realtime PCR
   - Make sure the synthetic siRNA has deoxythymine d(TT) 3′ overhangs.
   - Check the procedures for sample RNA preparation for any problems. Total RNA preparation kits that use a spin column generally are unsuitable for recovering low molecular weight RNAs such as synthetic siRNAs. When preparing RNA for the purpose of recovering synthetic siRNA, use RNAiso Plus or a commercially available small-RNA purification kit.
   - Check the sequence on the primer used for realtime PCR. Refer to Section VIII. 2 and make sure the primer is designed correctly.

2. Unsatisfactory PCR amplification efficiency
   - To obtain good quantitative results, be sure to perform heat denaturation of samples before the poly dA tailing reaction. The temperature for heat treatment differs depending on the siRNA sequence. Try another temperature between 70 and 80℃. To prevent re-annealing of the heat-denatured siRNA, do not leave on ice for longer than 15 minutes. Immediately carry out the subsequent poly dA tailing reaction.
   - Prepare serial dilutions using the target synthetic siRNA. Then use this product to generate cDNAs and carry out realtime PCR to confirm adequate PCR amplification efficiencies. A proper range is between 70 and 120% for the amplification efficiency determined here, which is subject to influence by poly dA tailing reaction and reverse transcription reaction.
   - After generating cDNA with this product from the target synthetic siRNA, prepare serial dilutions of cDNA and then carry out realtime PCR to confirm adequate PCR amplification efficiencies. The proper range for the amplification efficiency determined here is 80 to 120%.
   - The recommended realtime PCR reagent is TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/B), which is highly versatile and well balanced between amplification efficiency and reaction specificity. For templates that have somewhat difficult to amplify sequences, using TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/B) may improve the amplification efficiency.

3. Presence of nonspecific amplification products
   - Carry out a realtime PCR with a no-template control that contains no synthetic siRNA or cDNA to make sure there are no nonspecific amplifications.
   - It is important to take steps to avoid contamination between samples, such as starting with the sample containing the lowest concentration of synthetic siRNA when adding a reagent.
   - Some synthetic siRNAs have sequences that are prone to nonspecific amplifications.

XI. Related Products

< RNA extraction reagents, etc. >
RNAiso Plus (Cat. #9108/9109)
Dr. GenTLE™ Precipitation Carrier (Cat. #9094)

< Realtime PCR reagents/apparatus >
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B)

< A combination of this product (Cat. #6142) and RR820A >
Synthetic siRNA Quantitation Kit (with TB Green® *Premix Ex Taq*™ II Tli) (Cat. #RR861A)*

* Not available in all geographic locations. Check for availability in your area.