QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit

RMQ-101 96 reactions
RMQ-101T 24 reactions

Store at minus (-) 20℃

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.

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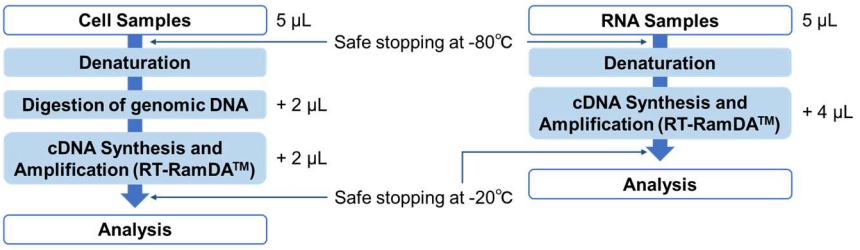
[1] Introduction

Description

The QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit (Code No. RMQ-101) is an improved version of the RT-RamDA™ cDNA Synthesis Kit (Code No. RMD-201) for preparing cDNA from single cells or trace amounts of RNA for real-time PCR analysis. Using this kit, cDNA can be prepared from full-length total RNA, and gene expression analysis can be performed with high sensitivity.

This kit uses reverse transcription with random displacement amplification (RT-RamDA™) (see method in reference (1)). RT-RamDA™ is a novel cDNA amplification method that utilizes the strand displacement activity of reverse transcriptase, which can detect not only poly(A) RNA but also non-poly(A) RNA with high sensitivity. For this reason, the RT-RamDA™ method is characterized by its ability to detect more genes than conventional technology.



Process Workflow

Features

1. cDNA can be prepared from a single cell or a small amount of input RNA

Between 1–500 cells or 10 pg–10 ng total RNA should be used.

2. Low-copy genes can be analyzed

By amplifying RNA to tens of times the amount of cDNA, low-copy genes can be analyzed from samples containing small amounts of RNA.

3. Very accurate quantification

Amplification occurs randomly in RT-RamDA™, which can reduce amplification bias.

4. Minimize input samples

Because amplified cDNA can be obtained, valuable samples are saved.

5. Can be used with a variety of real-time PCR reagents

This kit can be used in combination with a variety of real-time PCR reagents and is compatible with both SYBR® Green I and TaqMan® assays.

THUNDERBIRD® qPCR Mix (Code No. QPS-101, QPS-201, QPX-201), and KOD SYBR® qPCR Mix (Code No. QKD-201) can be used.

[2] Components

The kits include the following reagents that can be used for 96-well (RMQ-101) or 24-well (RMQ-101T) reactions. All reagents should be stored at -20℃.

Important: Do not store any of the solutions once mixed. All reagent cocktails should be prepared shortly before use.

QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit (Cat. No. RMQ-101 and RMQ-101T)

	RMQ-101	RMQ-101T
①Lysis Buffer	480 µL	120 µL
②Lysis Enhancer	108 µL	27 µL
③RNase Inhibitor	22 µL	6 µL
④RT-RamDA™ Buffer	160 µL	40 µL
⑤gDNA Remover	54 µL	14 µL
⑥RT-RamDA™ Enzyme Mix	60 µL	15 µL
⑦RT-RamDA™ Primer Mix	60 µL	15 µL
⑧Nuclease-free water	960 µL	240 µL

Required materials not included in the kit

Thermocycler

Equipment and reagents for real-time PCR

[3] Protocol

The kit can be used with cells or purified total RNA. Start with Section 1A when using FACS to obtain the cells, Section 1B when using non-FACS cells, and Section 1C when using purified total RNA. Thereafter, proceed with denaturation as outlined in Section 2.

Sample		Cell lysis or RNA dilution	Denaturation
Cells	FACS	Section 1-1. A (p. 1)	Continue with Section 1-2. (p. 2)
	Non-FACS	Section 1-1. B (p. 2)	
Purified RNA		Section 2-1. (p. 5)	Continue with Section 2-2. (p. 6)

1. When using cells

1-1. Cell lysis

A. *When acquiring cells by FACS*

(1) Prepare a cocktail of the cell lysis reagents in a single tube using the volumes listed in the table below. The volumes used will need to be adjusted depending on the number of reactions that you wish to perform.

Cell lysis cocktail

	1 Reaction (µL)	20 Reactions (µL)*	100 Reactions (µL)*
①Lysis Buffer	2	44	220
②Lysis Enhancer	0.45	9.9	49.5
③RNase Inhibitor	0.05	1.1	5.5
⑧Nuclease-free water	2.5	55	275
Total	5	110	550

*An extra 10% volume was added here to account for pipetting error.

(2) Dispense 5 µL of cell lysis cocktail per well into a 96-well plate or 8-tube strip. Dispense on ice, and immediately after dispensing, seal with a qPCR seal or thermocompression seal.

(3) Store the plate on ice or at 4℃ until cell sorting according to the FACS user manual and the parameters recommended by the manufacturer.

(4) After sorting, seal the plate or tubes and then centrifuge briefly to collect the solution at the bottom of the wells or tubes.

(5) Proceed immediately to the next step or store the samples at -80℃.

B. Cell acquisition using methods other than FACS

When obtaining the cells by manual picking, the volume of the cell sample should be 1.5 µL or less.

(1) Prepare the reagents necessary for cell lysis in one tube per reaction using the volumes listed in the table below, and then add the cell samples.

If the volume of the cell sample is less than 1.5 µL, adjust the amount of water added when preparing the cell lysis cocktail.

	1 Reaction (µL)
①Lysis Buffer	2
②Lysis Enhancer	0.45
③RNase Inhibitor	0.05
Sample	~1.5
⑧Nuclease-free water	X (adjust as needed based on sample volume)
Total	5

For example, if your cell sample volume is 1 µL, prepare the cell lysis cocktail using the volumes listed in the table below, and dispense 4 µL of the cell lysis cocktail per well into a 96-well plate or 8-strip tube. Then, add 1 µL of your cell samples.

Cell lysis cocktail

	1 Reaction (µL)	20 Reactions (µL) *	100 Reactions (µL) *
①Lysis Buffer	2	44	220
②Lysis Enhancer	0.45	9.9	49.5
③RNase Inhibitor	0.05	1.1	5.5
⑧Nuclease-free water	1.5	33	165
Total	4	88	440

*An extra 10% volume was added here to account for pipetting error.

(2) Seal the plate or tubes and then centrifuge briefly to collect the solution at the bottom of the wells or tubes.

(3) Proceed immediately to the next step or store the samples at -80℃.

1-2. Denaturation

(1) Centrifuge the sample plate or tubes at 4℃ and incubate them according to the table below.

Note: When using frozen samples, first thaw the samples at 4℃ and then briefly centrifuge them before proceeding to heat denaturation.

Step	Temperature	Time
Denature	75℃	1.5 min
Hold	4℃	∞

1-3. Digestion of genomic DNA

(1) Prepare a cocktail of the reagents necessary for genomic DNA removal using the volumes listed in the table below. The volumes used will need to be adjusted depending on the number of reactions that you wish to perform.

DNA removal cocktail

	1 Reaction (µL)	20 Reactions (µL)*	100 Reactions (µL)*
④RT-RamDA™ Buffer	0.2	4.4	22
⑤gDNA Remover	0.45	9.9	49.5
⑧Nuclease-free water	1.35	29.7	148.5
Total	2	44	220

*An extra 10% volume was added here to account for pipetting error.

(2) Add 2 µL of the genomic DNA removal cocktail per well to the 96-well plate or 8-tube strip containing the heat-denatured samples from the previous step. Centrifuge briefly, then incubate at the following temperatures:

Step	Temperature	Time
Genomic DNA digestion	30℃	5 min
Hold	4℃	∞

(3) Proceed immediately to the next step after gDNA digestion has occurred.

1-4. cDNA synthesis and amplification reaction (RT-RamDA™ reaction)

(1) Prepare a cocktail of the necessary reagents for the RT-RamDA™ reaction in a single tube using the volumes listed in the table below. The volumes used will need to be adjusted depending on the number of reactions that you wish to perform.

RT-RamDA™ cocktail

	1 Reaction (µL)	20 Reactions (µL)*	100 Reactions (µL)*
④RT-RamDA™ Buffer	1	22	110
⑥RT-RamDA™ Enzyme Mix	0.5	11	55
⑦RT-RamDA™ Primer Mix	0.5	11	55
Total	2	44	220

*An extra 10% volume was added here to account for pipetting error.

(2) Add 2 µL of RT-RamDA™ cocktail solution per well to the 96-well plate or 8-tube strips in which the genomic DNA removal reactions were performed. After brief centrifugation, incubate the plate or tubes at the following temperatures:

Step	Temperature	Time
Priming 1	25°C	10 min
Priming 2	30°C	10 min
Reverse transcription and amplification	37°C	30 min
	50°C	5 min
Inactivation	98°C	5 min
Hold	4°C	∞

(3) Proceed immediately to the next step after the RT-RamDA™ reaction, or store the plate or tubes at -20°C to -30°C.

(4) When performing real-time PCR, add the RT-RamDA™ reaction solution directly or diluted to the reaction solution as a template.
In real-time PCR, the template amount of the RT-RamDA™ reaction solution should be limited to less than 10% of the total volume. For example, if the total volume is 20 µL for real-time PCR, up to 2 µL of RT-RamDA™ reaction solution can be applied. The addition of a large amount of RT-RamDA™ reduces the efficiency of PCR, and accurate quantification may no longer be possible.

2. When using purified total RNA

2-1. RNA dilution

(1) Prepare the reagents necessary for RNA dilution in one tube per reaction using the volumes listed in the table below and then add the cell samples.
If the volume of the cell sample is less than 2.5 µL, adjust the amount of water.

	1 Reaction (µL)
①Lysis Buffer	2
②Lysis Enhancer	0.45
③RNase Inhibitor	0.05
Sample	~2.5
⑧Nuclease-free water	X (adjust as needed based on sample volume)
Total	5

For example, if the RNA sample volume is 2 µL, prepare the RNA dilution cocktail using the volumes listed in the table below, and dispense 3 µL of the RNA dilution cocktail per well into a 96-well plate or 8-strip tube. Then, add 2 µL of the RNA samples.

RNA dilution cocktail

	1 Reaction (µL)	20 Reactions (µL) *	100 Reactions (µL) *
①Lysis Buffer	2	44	220
②Lysis Enhancer	0.45	9.9	49.5
③RNase Inhibitor	0.05	1.1	5.5
⑧Nuclease-free water	0.5	11	55
Total	3	66	330

*An extra 10% volume was added here to account for pipetting error.

(2) After dispensing, seal the tubes and centrifuge briefly to collect the solutions at the bottom of the wells or tubes.

(3) Proceed immediately to the next step or store the samples at -80°C.

2-2. Denaturation

(1) Centrifuge the sample plate or tubes at 4℃ and incubate as described in the table below. Note: When using frozen samples, first thaw the samples at 4℃, and then proceed to heat denaturation after brief centrifugation.

Step	Temperature	Time
Denature	75℃	1.5 min
Hold	4℃	∞

2-3. cDNA synthesis and amplification reaction (RT-RamDA™ reaction)

(1) Prepare a cocktail of reagents necessary for the RT-RamDA™ reaction using the volumes listed in the table below. The volumes used will need to be adjusted depending on the number of reactions that you wish to perform.

RT-RamDA™ cocktail

	1 Reaction (μL)	20 Reactions (μL)*	100 Reactions (μL)*
④RT-RamDA™ Buffer	1.2	26.4	132
⑤gDNA Remover	0.45	9.9	49.5
⑥RT-RamDA™ Enzyme Mix	0.5	11	55
⑦RT-RamDA™ Enzyme Mix	0.5	11	55
⑧Nuclease-free water	1.35	29.7	148.5
Total	4	88	440

*An extra 10% volume was added here to account for pipetting error.

(2) Add 4 μL of the RT-RamDA™ cocktail per well to the 96-well plate or 8-tube strip containing the heat-denatured samples from the previous step. Centrifuge briefly, and then incubate at the following temperatures:

Step	Temperature	Time
Priming 1	25℃	10 min
Priming 2	30℃	10 min
Reverse transcription and amplification	37℃	30 min
Reverse transcription 2	50℃	5 min
Inactivation	98℃	5 min
Hold	4℃	∞

(3) Proceed immediately to the next step after the RT-RamDA™ reaction or store the plate or tubes at -20℃ to -30℃.

(4) When performing real-time PCR, add the RT-RamDA™ reaction solution directly or diluted to the reaction solution as a template.
In real-time PCR, the template amount of the RT-RamDA™ reaction solution should be limited to less than 10% of the total volume. For example, if the total volume is 20 µL in real-time PCR, up to 2 µL of RT-RamDA™ reaction solution can be applied.
The addition of a large amount of RT-RamDA™ reduces the reaction efficiency of the PCR, and accurate quantification may no longer be possible.

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[4] Application data

Application 1. Comparison of the amount of cDNA between the QuantAccuracy™ and conventional reverse transcription kits

<Method>

cDNA synthesis was performed using 1 ng of total RNA extracted from HeLa S3 cells to compare the QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit (QuantAccuracy, Code No. RMQ-101) with a conventional reverse transcription kit from competitor A. Real-time PCR analysis was then performed on two types of long non-coding RNA genes (*NEAT1* and *MALAT1*) and seven types of housekeeping genes (*ACTB*, *GAPDH*, *ATP5F1A*, *YWHAZ*, *PPIA*, *B2M*, *RPS18*, and *HPRT1*) using THUNDERBIRD® SYBR® qPCR Mix (Code No. QPS-201). Each cDNA was used as a template in a 20 µL reaction volume. The amount of cDNA obtained using the QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit was calculated from the Ct value, and the value obtained by dividing by the amount of cDNA in the conventional reverse transcription reaction was calculated as the amplification rate.

<Results>

There was a significantly high correlation between the Ct values of the QuantAccuracy™ and reverse transcription kits (Figure 1). In addition, the average ΔCt values of the genes between the QuantAccuracy™ and reverse transcription kits were > 5 (Figure 2). Thus, by using the QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit, it is possible to obtain 30 times or more cDNA than that obtained using the conventional reverse transcription reaction reagent.

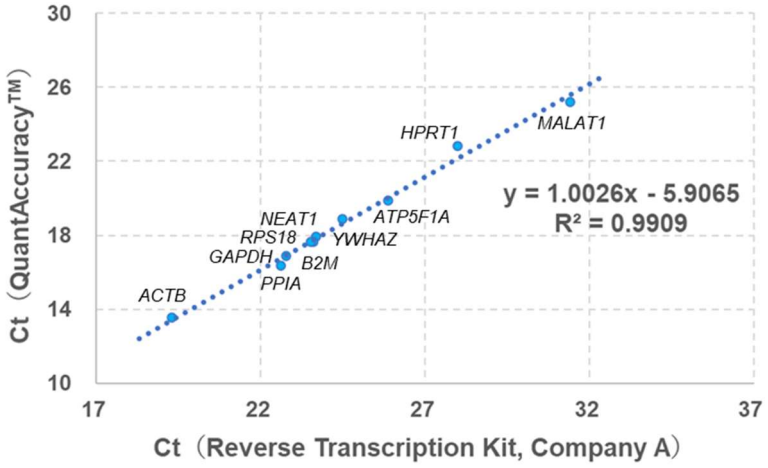

Figure 1. Correlation between QuantAccuracy™ (y-axis) and a conventional reverse transcription kit (x-axis, Company A).

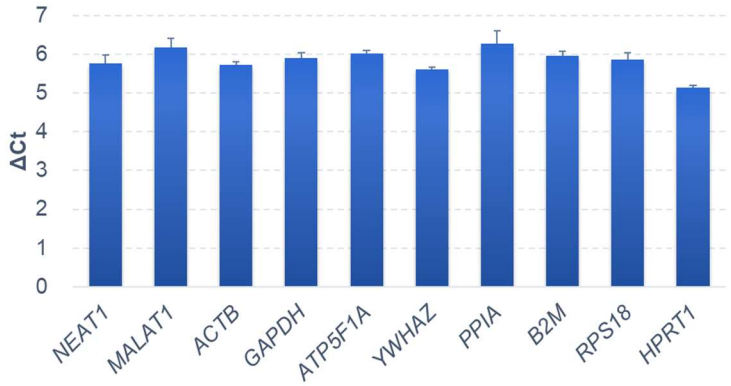

Figure 2. ΔCt values between QuantAccuracy™ and a conventional reverse transcription kit

Application 2. Analysis using a human FFPE tissue

<Method>

cDNA synthesis was performed using 100 pg or 1 ng RNA extracted from human FFPE tissue to compare the QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit (Code No. RMQ-101) with other cDNA amplification kits from competitors B and C, and a conventional reverse transcription kit from competitor D. As in Application 1, real-time PCR analysis was performed on two types of long non-coding RNA genes and seven types of housekeeping genes using THUNDERBIRD® SYBR® qPCR Mix (Code No. QPS-201) with each cDNA used as a template.

The amount of cDNA in the QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit was calculated from the Ct value, and the value obtained by dividing by the amount of cDNA in the conventional reverse transcription reaction was calculated as the amplification rate.

<Results>

First, compared to the other products, QuantAccuracy™ exhibited the highest detection rate for all genes (Table 1).

In addition, among these products, only QuantAccuracy™ yielded parallel line graphs connecting Ct values of the genes in the study with 100 pg and 1 ng FFPE RNA, indicating that constant amplification occurred irrespective of the type of genes (Figure 3). The average ΔCt value in the study using QuantAccuracy™ was calculated to be approximately 3.40, indicating that the 10-fold difference between FFPE RNAs was $2^{3.40} \approx 10.6$ times difference (Figure 3). Thus, the QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit allows highly accurate analysis despite the use of FFPE tissues n this study.

Table 1. Detection rates of the genes

	Quant Accuracy™	cDNA Amplification Kit, Company B	cDNA Amplification Kit, Company C	Reverse Transcription Kit, Company D
NEAT1	100%	100%	0%	100%
MALAT1	50%	17%	0%	17%
ACTB	100%	67%	50%	100%
GAPDH	100%	50%	33%	100%
ATP5F1A	100%	17%	0%	83%
YWAHZ	100%	50%	33%	100%
PPIA	100%	33%	0%	100%
B2M	100%	100%	100%	100%
RPS18	100%	17%	50%	100%
HPRT1	50%	17%	50%	33%

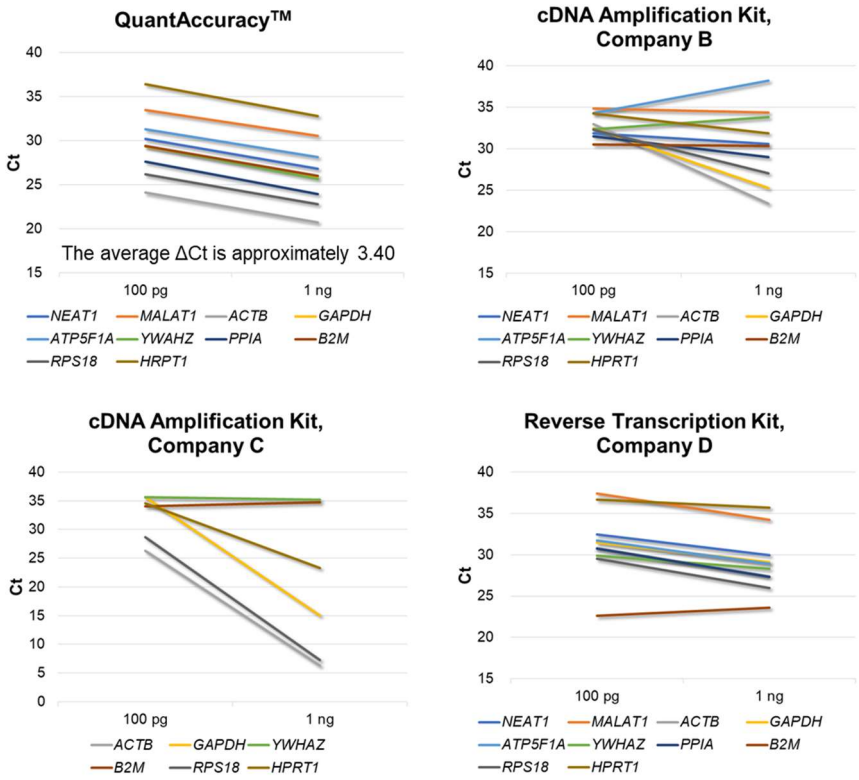

Figure 3. Line graphs connecting the Ct values of the genes in the study with 100 pg and 1 ng FFPE RNA

[5] Troubleshooting

Symptom	Cause	Solution
No signal or delayed detection in real-time PCR	RNA is degraded	· Check whether RNA has degraded. · Check whether RNase has contaminated the tips or tubes used. · Perform all cell lysis and RNA dilution steps on ice.
	Excess reverse transcription reaction solution added	If excess amount is added, PCR may be inhibited. The amount of reverse transcription reaction solution added to the real-time PCR reaction solution should be 10% or less of the amount of PCR solution.

[6] Related products

Product name	No. of reactions	Catalog No.
GenNext™ RamDA-seq ™ Single Cell Kit	96	RMD-101
cDNA prep kit for NGS	24	RMD-101T
RT-RamDA™ cDNA Synthesis Kit	96	RMD-201
	24	RMD-201T
THUNDERBIRD® Probe qPCR Mix	500	QPS-101
THUNDERBIRD® SYBR® qPCR Mix	500	QPS-201
THUNDERBIRD® Next SYBR® qPCR Mix	500	QPX-201
KOD SYBR® qPCR Mix	500	QKD-201

Reference 1

Tetsutaro Hayashi*, Haruka Ozaki*, Yohei Sasagawa, Mana Umeda, Hiroki Danno and Itoshi Nikaido. Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. Nature Communications. 2018.