New

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TOYOBO

cDNA Synthesis kit for Real-time PCR analysis

QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit

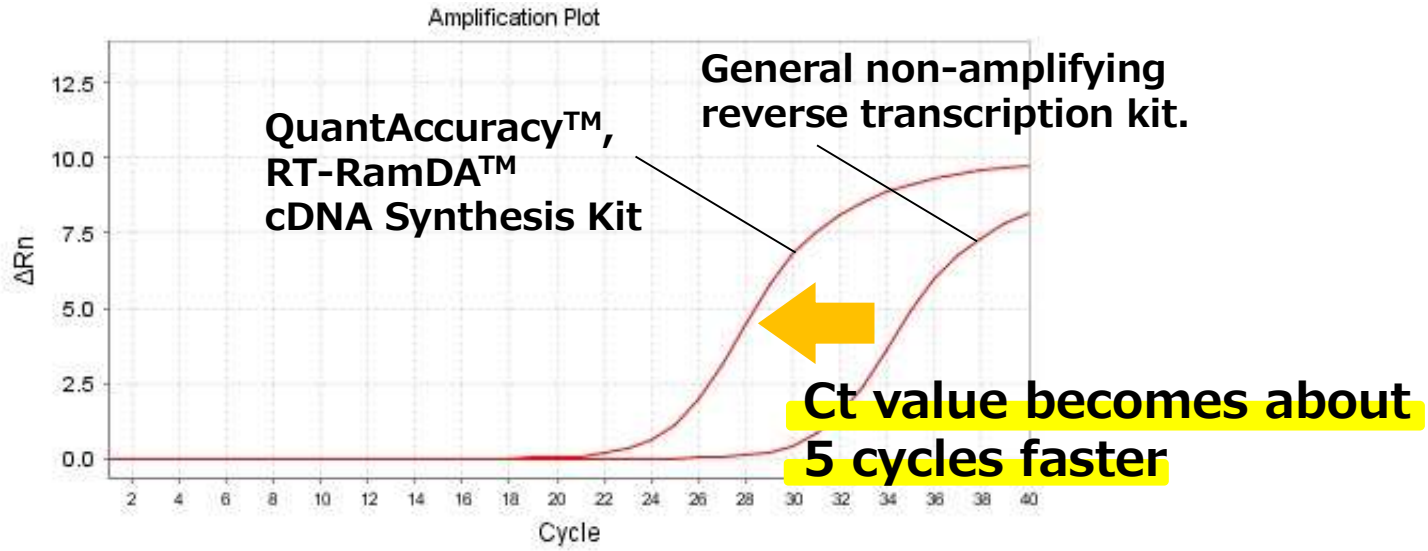
Single cell

Low copy RNA

FFPE

The RT-RamDA™ method, a new cDNA amplification method, enables cDNA synthesis from trace RNA*

***Compatible with 1 to 500 cells or 10 pg to 10 ng total RNA**



This is the example of preparing cDNA for purified RNA 10pg derived from HeLa cells using non-amplified reverse transcriptase or this product, and amplifying *ACTB* by real-time PCR.

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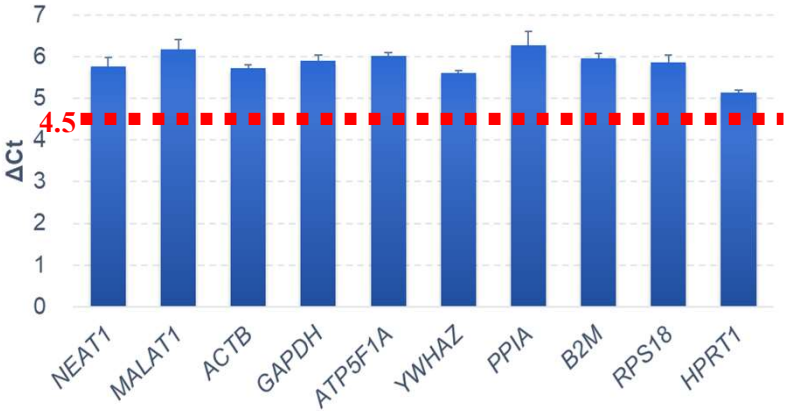
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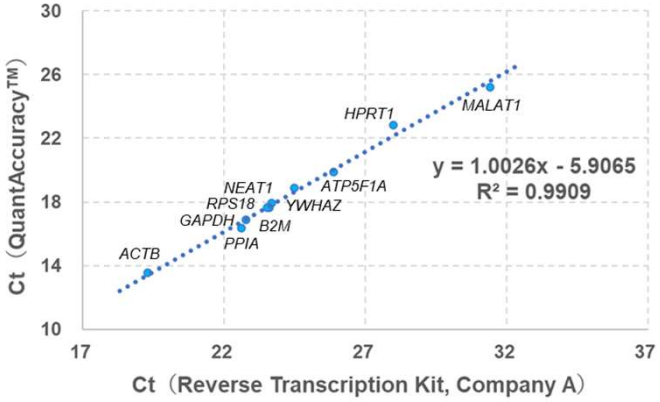
QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit can obtain 20 times more cDNA than general non-amplified reverse transcription kit.

- **Can prepare cDNA from single cell or trace RNA!**
- **Increased number of detected genes from the same total RNA amount!**
- **Valuable RNA samples can be analyzed with a small amount of input!**

ΔCt values between QuantAccuracy™ and a conventional reverse transcription kit



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

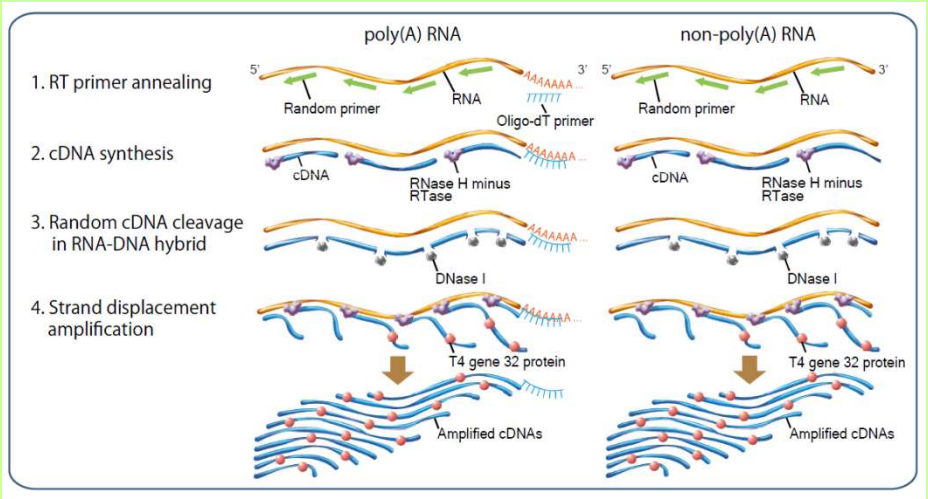


cDNA synthesis was performed using 1 ng of total RNA extracted from HeLa S3 cells to compare the QuantAccuracy™, RT-RamDA™ cDNA Synthesis Kit (Code No. RMQ-101) with a conventional reverse transcription kit from Company A.
Real-time PCR analysis was then performed on two types of long non-coding RNA genes (*NEAT1* and *MALAT1*) and eight types of housekeeping genes.
As a result, a correlation of Ct value was observed between this product and the reverse transcription reagent without amplification ability, and ΔCt> 4.5 for all genes, and it was confirmed that this product has an amplification factor of $2^{4.5}$≒20 times or more.

RT-RamDA™

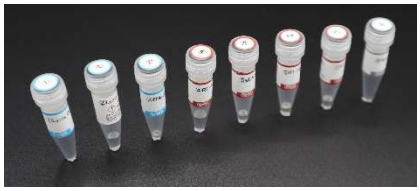
RT-RamDA ™ is an abbreviation for "Reverse Transcription with Random Displacement Amplification" developed by the Bioinformatics Research and Development Team, RIKEN Center for Biosystems Science and Technology.It is a new cDNA amplification method that applies the strand substitution activity of reverse transcriptase, and it is possible to prepare cDNA not only from poly (A) RNA but also from non-poly (A) -derived RNA. Since the cDNA is amplified at the same time as synthesis, it is possible to suppress the bias due to amplification without the need for addition of an amplification adapter or PCR, which is performed by the conventional method.

*Hayashi .T et al. Single-cell full-length total RNA sequencing uncovers dynamics of recursive splicing and enhancer RNAs. Nature Communications. 9 :619(2018)



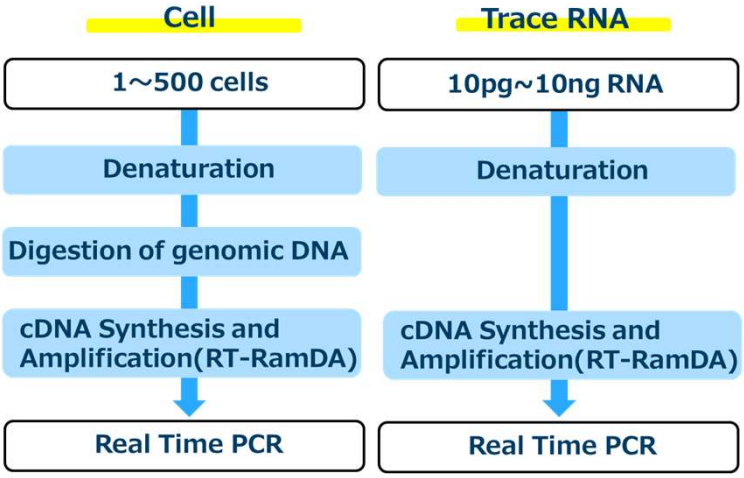
Parts of this product





①Lysis Buffer
②Lysis Enhancer
③RNase Inhibitor
④RT-RamDA™ Buffer
⑤gDNA Remover
⑥RT-RamDA™ Enzyme Mix
⑦RT-RamDA™ Primer Mix
⑧Nuclease free water

Process workflow



The prepared cDNA can be stored at -20 ° C.

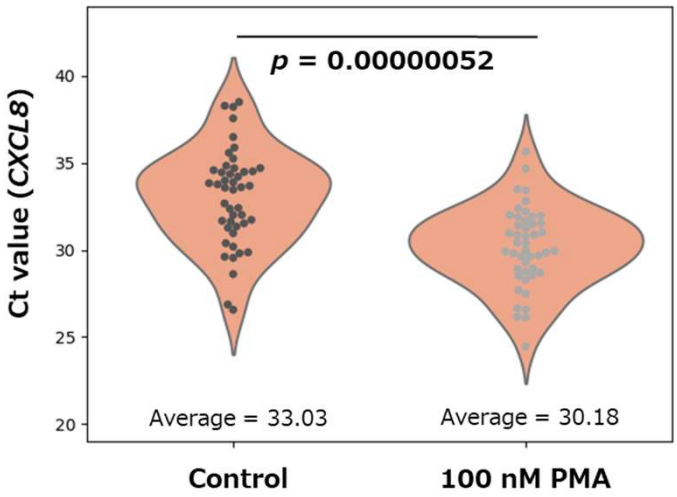
Application 1 Preparation of cDNA from a single cell

● Method

Through Phorbol 12-myristate 13-acetate (PMA) treatment, a single-cell expression analysis experiment of the inflammatory cytokine gene *IL-8* was performed. HeLa S3 cells were treated with 100 nM PMA for 24 hours, and then the cells were separated one by one using a cell sorter. cDNA was prepared from one separated cell (n = 48) using this product, and the *IL-8 (CXCL8)* gene was detected using THUNDERBIRD™ SYBR™ qPCR Mix (Code No. QPS-201).

● Result

It was found that the Ct value was reduced by about 3 by PMA treatment and the expression level of *IL-8* was significantly increased (p value was calculated from Student's t-test).By using this product, it was possible to analyze gene expression even in a single cell level.



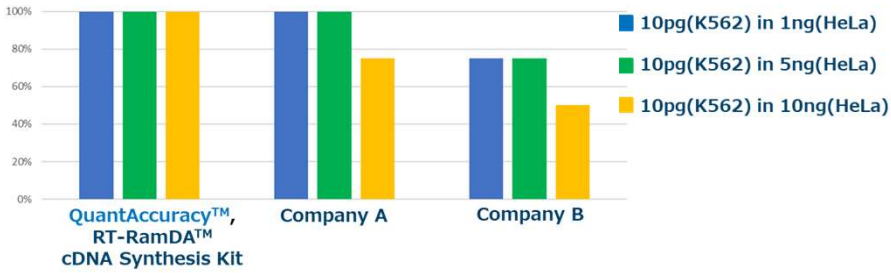
Application 2 Preparation of cDNA from trace RNA

● Method

Genes that are thought to be the causative genes of chronic leukemia were investigated.10 pg of K562 cell-derived RNA (*BCR-ABL* (+)) was spiked into 1 ng, 5 ng, and 10 ng HeLa cell-derived RNA (*BCR-ABL* (-)). Then, cDNA was prepared using each reverse transcription reagent, and the *BCR-ABL* gene was detected by real-time PCR using THUNDERBIRD™ SYBR™ qPCR Mix (Code No. QPS-201) (n = 4).

● Result

As the amount of RNA derived from HeLa cells increased, the efficiency of cDNA synthesis from trace of RNA decreased with the reverse transcription reagent without amplification ability, and detection omission occurred. Our products were able to efficiently prepare cDNA and all were detectable.



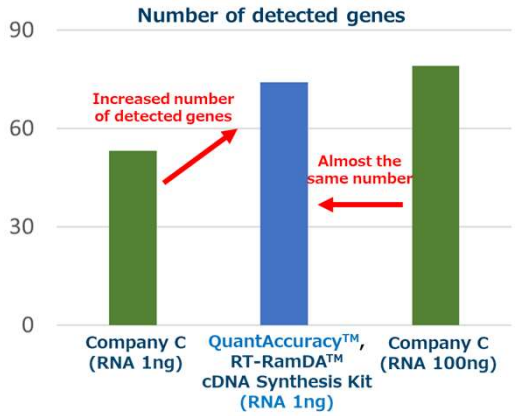
Application 3 Input amount and number of detected genes

Method

cDNA was prepared from iPS cell-derived RNA (1 ng, 100 ng) using each reverse transcription reagent. Then, it was added to TaqMan Array Mouse Stem Cell Pluripotency, and 96 kinds of genes were detected by THUNDERBIRD™ Probe qPCR Mix (Code No. QPS-101).

Result

In the input amount of RNA1ng, this product was able to detect more genes than the reagent of Company C. In addition, almost the same number of genes were detected as when cDNA was prepared from 100 times the amount of RNA (100 ng) using the company C reverse transcription reagent.



Application 4 Preparation of cDNA from FFPE-derived RNA

Method

cDNA was prepared from purified RNA (100 pg, 1 ng) derived from human FFPE specimens using each reverse transcription reagent. Then, detection and quantitative analysis of 2 nuclear RNA genes (*NEAT1, MALAT1*) and 8 housekeeping genes (*ACTB, GAPDH, ATP5F1A, YWHAZ, PPIA, B2M, RPS18, HPRT1*) were performed.THUNDERBIRD™ SYBR™ qPCR Mix (Code No. QPS-201) was used as the real-time PCR reagent, and 1 µL of cDNA solution was input to the 20 µL reaction system (n = 6).

Result

The detection rates of each gene from 100 pg RNA were compared. As a result, cDNA prepared using this product showed the highest detection rate for any of the genes.cDNA was prepared using different RNA amounts (100 pg, 1 ng) as a template, amplified by real-time PCR, and the Ct values were compared.As shown in the figure below, the Ct value when using 100 pg RNA and the Ct value when using 1 ng RNA are connected by a straight line. There was almost no difference in amplification rate between each gene only when this product was used.In addition, the average ΔCt value between 100pg and 1ng of this product was about 3.40.In other words, in the analysis using this product, the difference in the amount of RNA 10 times can be evaluated as the difference in the amounts of gene $2^{3.4} \fallingdotseq 10.6$ times.For RNA derived from FFPE specimens, this product can be analyzed with little bias in amplification factor.

	QuantAccuracy™ [Amplification ability (+)]	Company D [Amplification ability (+)]	Company E [Amplification ability (+)]	Company F [Amplification ability (-)]
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%

