DTI One Step FabScript II RT-qPCR premix

Instruction Manual for use

User Manual for DTI One Step FabScript II RT-qPCR premix

Catalogue number: DT0701.200

Note: Applicable to all pack sizes

Manufactured by
DSS Takara Bio India Pvt Ltd
A-5 Mohan Co-operative, Industrial Estate,
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1) Product Description:

DTI One Step FabScript II RT-qPCR premix is a dedicated reagent for one-step real-time, probe-based RT-qPCR (using the 5' nuclease method). This 2X premix does not freeze at its storage temperature of -20℃, so a reaction can be started simply by adding the template sample, primer, and a probe for detecting the desired target. The quick and simple protocol allows the reverse transcription and qPCR reactions to be performed in the same tube.

The reverse transcription reaction uses the novel FabScript II RTase, which displays increased heat tolerance (up to 55℃) while maintaining the specificity and extensibility of FabScript RTase. This allows cDNA synthesis from RNA with a more complex secondary structure. After cDNA synthesis, DTI FabTaq HS performs highly specific and efficient PCR amplification, while the fluorescence emitted by the probe is detected in real-time.

DTI One Step FabScript II RT-qPCR premix is also highly resistant to a wide variety of inhibitory substances such as heparin (blood) and humic acid (soil), allowing stable one-step real-time RT-qPCR to be performed on a wide range of samples. This product can be used for various applications such as gene expression, RNA virus detection, etc.

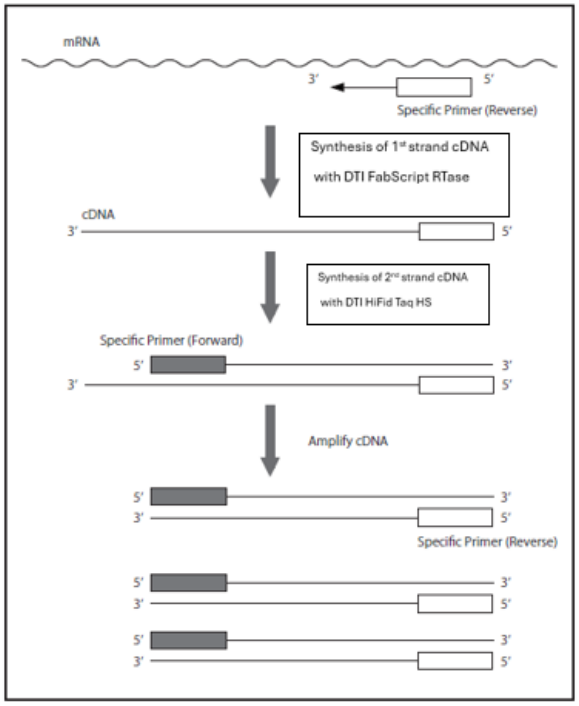
2) Principle

DTI One Step FabScript II RT-qPCR premix allows reverse transcription using FabScript II RTase and qPCR using DTI FabTaq HS to be performed in the same tube. PCR amplification products are monitored by a probe in real-time.

i. RT-PCR

Although RNA does not serve as a direct template for PCR, synthesizing cDNA from RNA using reverse transcriptase allows PCR to be used for RNA analysis. This highly sensitive RNA detection method is known as RT-PCR. This product performs one step RT-PCR, as shown in the figure on the following page.

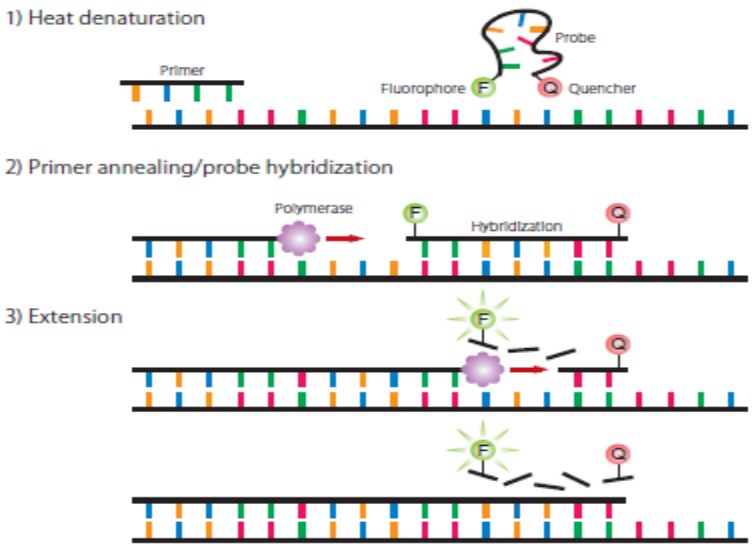
In one step RT-PCR, a reverse transcription reaction is performed using a specific primer (reverse) for PCR and then PCR amplification is performed by specific primers (forward, reverse) using synthesized cDNA as a template. Both steps are performed in the same tube.



Principle of One Step RT-PCR method

ii. Fluorescence Detection

This reagent uses a detection probe that is an oligonucleotide whose 5' and 3' ends are modified with a fluorescent substance (FAM, etc.) and a quencher substance (TAMRA, BHQ1, etc.), respectively. Under the annealing conditions, the probe specifically hybridizes to a template DNA, but the fluorescence is suppressed by the quencher. At the time of the extension reaction, however, the probe hybridizing to the template is degraded by the 5' → 3' exonuclease activity of Taq DNA polymerase and the suppression by the quencher disappears. Fluorescence produced in this process is detected by a real-time PCR machine. A method combining the above principles enables sample quantitation at real-time, therefore it is called One Step RT-q (quantitative) PCR.



3) Components (200 reactions, 25 µl per reaction)

DTI One Step FabScript II RT-qPCR premix (2X)	625 µl x 4
RNase Free H2O	1.25 ml x 2
ROX Reference Dye (50X conc.)*1	100 µl

*1 It is added when a machine that performs fluorescence signal correction between wells such as a real-time PCR machine made by Applied Biosystems, etc. is used.

- ♦ An example of a machine where ROX Reference Dye is added:
  - Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific)
- ♦ Machines not requiring addition:
  - LightCycler series (Roche Diagnostics)
  - CFX series (Bio-Rad).

4) Materials required but not provided:

i. Gene amplification system for real-time PCR
[Thermal cyclers compatible with this product]
- Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific)
- LightCycler 96 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad)

ii. Dedicated reaction tube or plate
iii. Primers for PCR
iv. Probe for detection
v. Micropipette and tips

5) Storage

-20°C

6) Features

i. One-step RT-qPCR reagent for probe detection.
ii. Simple and quick protocol using a 2X premix that does not freeze at its storage temperature of -20℃.
iii. Reverse transcription at high temperatures (up to 55℃) is enabled by using a novel heat-resistant reverse transcriptase, FabScript II RTase.
iv. Highly resistant to inhibitory substances.
v. High reproducibility.

7) Precautions

Read these precautions before use and follow them when using this product.

i. Mix gently DTI One Step FabScript II RT-qPCR premix (2X) by inverting the tube and centrifuge quickly the tube to remove the solution attached to the tube lid before use. Immediately store it at -20℃ after use. If the product is frozen, it may be thawed and used without any loss of

quality. When white turbidity may be observed during storage, the product can be used after mixed evenly by inverting the tube then centrifuge quickly it to remove the solution attached to the tube lid before use.

ii. When reagents are dispensed, always use a new disposable tip, and avoid contamination between samples.

iii. For the reaction mixture, it is convenient to prepare a required amount+ α of Master Mix (a mixture of DTI One Step FabScript II RT-qPCR premix (2X), RNase -free H2O, and primer/probe or RNA sample). Data dispersion between experiments resulting from reagent preparation can be minimized by dispensing the minimum number of aliquots required from a Master Mix with a uniform composition.

iv. The reverse transcription reaction performed with this kit uses specific primers. Random Primer and Oligo dT Primer cannot be used.

8) Precautions during use

The DTI FabTaq HS that is used in this product is a hot-start PCR enzyme utilizing anti-Taq antibody that suppresses polymerase activity. Do not perform the 5 - 15 minute activation step at 95℃ before the PCR reaction that is required for other companies' chemically modified hot-start PCR enzymes. Unnecessary heat treatment decreases enzyme activity and can affect amplification efficiency and quantitation accuracy. Generally, 95℃ for 10 sec is sufficient for heat inactivation of reverse transcriptase before the PCR reaction.

9) Protocol

Follow the instructions in the user manual for each machine. For information regarding the RNA preparation method, refer to <Appendix: RNA sample preparation>.

A. Preparation of reaction mixture

Prepare a PCR reaction mixture as indicated below on ice and add 25 µl the reaction mixture in a tube or a well.

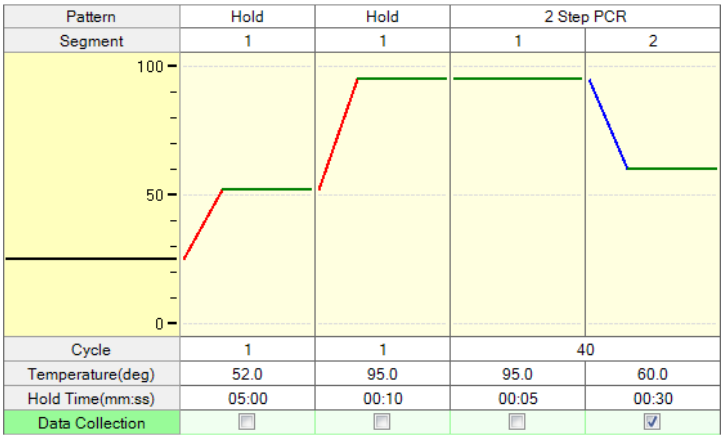
<For 1 reaction>

Reagent	Volume	Final conc.
DTI One Step FabScript II RT-qPCR premix (2X)	12.5 µl	1X
PCR Forward Primer (10 µM)	0.5 µl	0.2 µM[*1]
PCR Reverse Primer (10 µM)	0.5 µl	0.2 µM[*1]
Probe (10 µM)	0.5 µl	0.2 µM[*1]
RNA sample[*3]	≤2.5 µl	
RNase Free H2O	X µl[*4]	
Total	25 µl	

B. RT-qPCR reaction

After gently spin down the reaction tube or plate, place it in the Thermal Cycler Dice Real Time System and start the reaction under the following conditions. The recommended protocol for PCR reactions is the standard protocol described below. Try this protocol first and then

optimize the PCR reaction conditions as necessary. (Refer to <Determining RT-qPCR reaction conditions>.)



Pattern 1: Reverse transcription reaction

52℃ 5 min

95℃ 10 sec

Pattern 2: PCR reaction

Cycle: 40

95℃ 5 sec

60℃ 30 sec

C. After the reaction is complete, assess the amplification curve and create a standard curve if a quantitative determination will be performed.
For analytical methods, refer to the manual for the real-time PCR instrument used.

* 1 to 4: Check <Determining RT-qPCR reaction conditions>

Determining RT-qPCR reaction conditions

Reverse transcription reaction

Step	Temp.	Time	Detection	Comment
Reverse transcription	42 - 55℃	5 min	OFF	Improvement can be seen in some cases when the temperature is adjusted depending on the target.
Denaturation	95℃	10 sec	OFF	Generally, 95℃ for 10 sec is enough for heat inactivation of reverse transcriptase.

PCR reaction 30-45 cycles

Step	Temp.	Time	Detection	Comment
Denaturation	95℃	3 - 5 sec	OFF	Generally the amplification product size for real-time PCR does not exceed 300 bp. Therefore, 95℃ for about 3 to 5 sec is usually sufficient
Annealing/ Extension	56 - 64℃	20 - 30 sec[*5]	ON	When optimizing reaction conditions, evaluate results using an annealing/ extension temperature in the range of 56℃ to 64℃. If poor reactivity occurs, increasing incubation time for this step may improve results.

*1 A final primer concentration of 0.2 µM works well in most cases. However, should further optimization be required, try adjusting primer concentrations in the range of 0.1 to 1.0 µM.

*2 The probe concentration varies depending on the model of real-time PCR instrument used and the fluorescent labelling dye of the probe. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration. When using the Thermal Cycler Dice Real Time System, generally, try a final concentration in the range of 0.1 to 0.5 µM.

*3 The preferred sample size is between 10 pg and 1 µg of RNA in 1/10 or less of the reaction volume. Although 1/10 or more of the reaction volume can be used with a low target RNA concentration, this may inhibit the RT-qPCR reaction in some cases.

*4 Adjust the reaction volume according to the recommendations for the real-time PCR instrument used.

*5 Depending on the real-time PCR instrument used, it may not be possible to set the detection step within 30 sec. In that case, use a settable time for the instrument (31 or 34 sec, etc.).

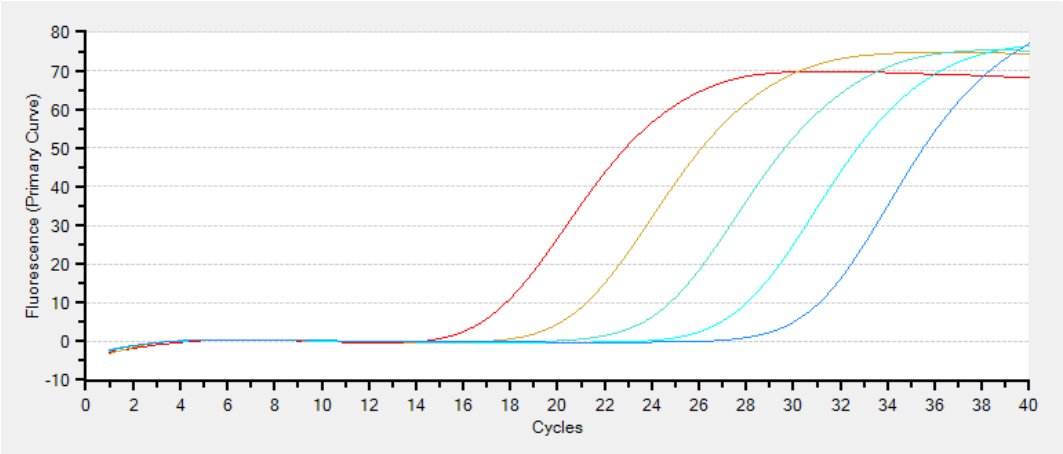
*6 For guidelines regarding the use of ROX Reference Dye, refer to the tables below.

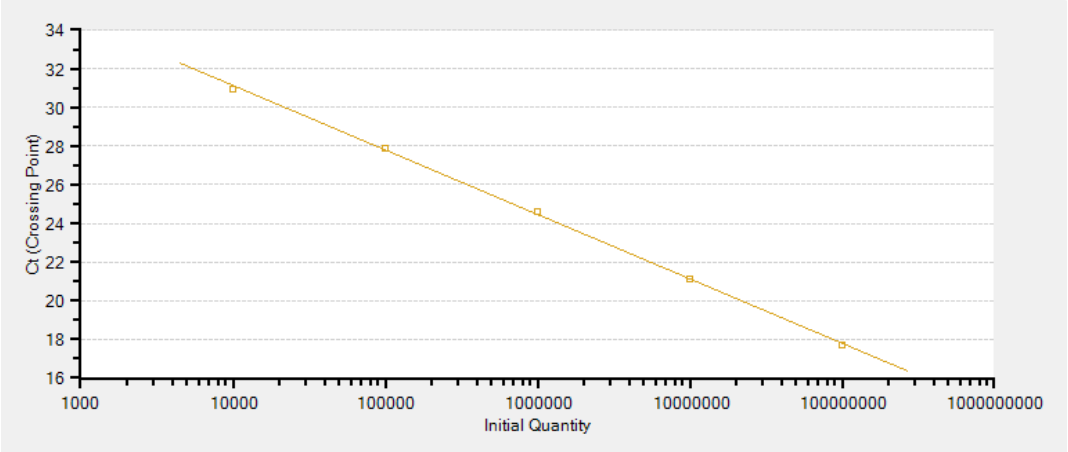
ROX Reference Dye (50X)
StepOne, StepOnePlus ABI 7300/7700/7900HT

Real-time PCR instruments that do not use ROX Reference Dye
CFX96, CFX384, Lightcycler

10) Experimental example

A consistent and quantitative detection of the human GAPDH expression was confirmed by using a serial dilutions of the total RNA as template. No expression was confirmed without the template. The product demonstrates an amplification efficiency between 90-110% and an R2 value of 0.99. Below are the images of amplification plots and standard curve.





11) Appendix : RNA sample preparation

This product is a kit for performing cDNA synthesis and PCR amplification from RNA. In order to synthesize cDNA successfully, it is essential to inhibit RNase activity in samples and avoid RNase contamination of equipment and solutions. Additional precautions should be taken during sample preparation, such as using clean disposable gloves and setting aside a designated area exclusively for RNA preparation.

[Equipment]

Disposable plastic equipment should be used whenever possible.

[Solutions]

All reagents and purified water should be used exclusively for RNA experiments.

[RNA preparation method]

This product is optimized to be highly resistant to a wide variety of inhibitory substances that may be present in PCR reactions using RNA samples obtained by using a simple nucleic acid extraction method. However, use of highly pure RNA is recommended when more highly reproducible results are required. NucleoSpin RNA (Cat. #740955.50/.250) provides a convenient spin column method for obtaining high-purity total RNA from cultured cells and tissue samples.

12) Related Products

RNase-free Water (Cat. #9012)
NucleoSpin RNA (Cat. #740955.50/.250)
NucleoSpin RNA Plus (Cat. #740984.50/.250)
NucleoSpin RNA Virus (Cat. #740956.50/.250)
DTI qPCR Dilution buffer

Visit https://store.dsstakarabio.com/pages/dti-one-step-fabscript-rt-qpcr-premix for more detailed product information

For more information contact directly below;
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Email: enquiries@dsstakarabio.com
Toll-Free number 1800-212-4922

Description of Symbol Used:

Catalogue number

Batch Code


Date of Manufacturing


Use-by-date


Contains sufficient for <n> tests