DTI Probe HiFid Taq HS Premix

Instruction Manual for use

User Manual for DTI Probe HiFid Taq HS Premix

Catalogue number: DT0603.100, DT0603.500

Note: Applicable to all pack sizes

Manufactured by
DSS Takara Bio India Pvt Ltd
A-5 Mohan Co-operative, Industrial Estate,
Mathura Road, New Delhi, Delhi 110044

1) Product Description:

DTI Probe HiFid Taq HS Premix is designed for probe-based qPCR. This product is suitable for high-speed PCR. DTI HiFid Taq allows accurate target quantification and detection over a broad dynamic range and makes it possible to conduct highly reproducible and reliable real-time PCR analyses.
The product is supplied as a 2X premix to facilitate easy preparation of reaction mixtures. The 2X premixed reagent also contains Tli RNaseH, a heat-resistant RNase H, to minimize PCR inhibition by residual mRNA in reactions using cDNA templates. A combination of DTI HiFid Taq HS polymerase, a hot-start PCR enzyme that uses an anti-Taq antibody, and a buffer optimized for real-time PCR suppresses non-specific amplification and allows high amplification efficiency and high detection sensitivity in real-time PCR analyses.

Advantages:

I) This product allows rapid and accurate detection and quantitative gene expression analysis by real-time PCR.
II) The premixed reagent simplifies reaction set up.
III) Includes DTI HiFid Taq HS polymerase, an enzyme designed for hot-start PCR. The buffer system has been optimized for real-time PCR and provides excellent amplification efficiency and highly sensitive detection.
IV) The 2X premixed reagent includes Tli RNaseH, a heat-resistant RNase H, to minimize PCR inhibition by residual mRNA in reactions using cDNA templates.

Compatible instruments:

- Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System and StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- LightCycler/LightCycler 480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad)
- Smart Cycler System/Smart Cycler II System (Cepheid)

2) Principle

This product uses DTI HiFid Taq HS for PCR amplification. PCR amplification products may be monitored in real time using a probe.

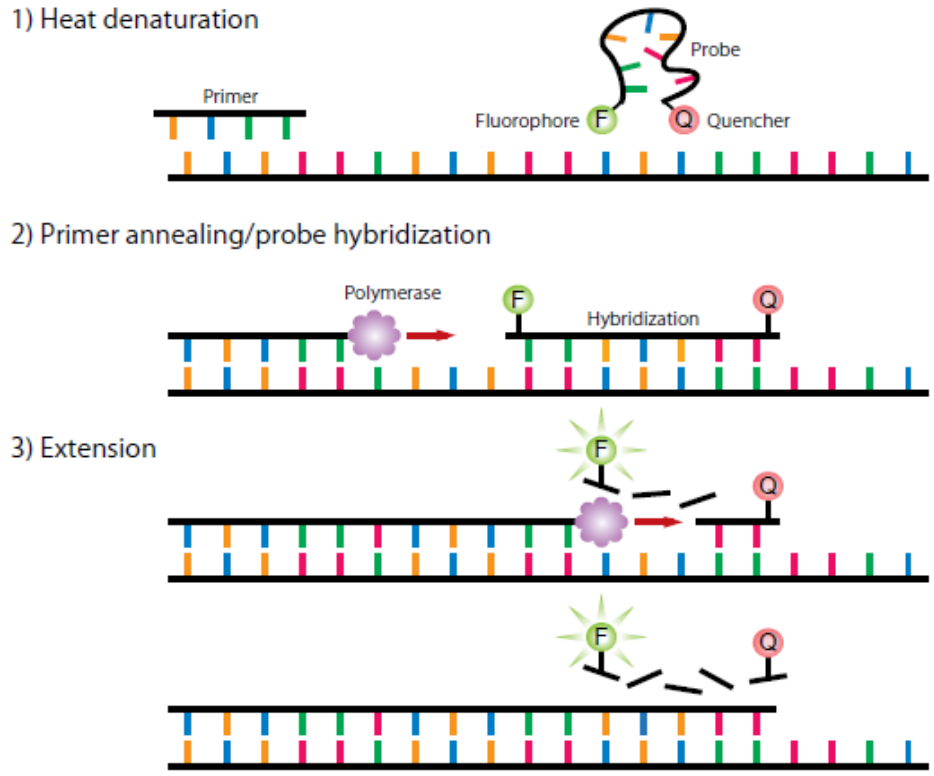
i. PCR

PCR is a technique used to amplify specific target sequences from minute amounts of DNA. By repeating three cycles of heat denaturation, primer annealing, and primer extension, the target fragment is amplified up to a million times by DNA polymerase within a short time.
This product uses DTI HiFid Taq HS, a hot-start PCR enzyme that prevents nonspecific amplification resulting from mispriming or primer dimer formation during reaction mixture preparation or other pre-cycling steps thereby allowing high sensitivity detection.

ii. Fluorescence Detection

Oligonucleotides modified with a 5' fluorophore (e.g., FAM) and a 3' quencher (e.g., TAMRA) are added to the reaction.

Under annealing conditions, the probe hybridizes in a sequence-specific manner to the template DNA. Fluorescence of the fluorophore is suppressed by the quencher. During the extension reaction, the 5'→ 3' exonuclease activity of Taq DNA polymerase degrades the hybridized probe, releasing quencher suppression and allowing fluorescence.



3) Components (20 µl per reaction)

Components	Cat# DT0603.100 (100 rxn)	Cat# DT0603.500 (500 rxn)
DTI Probe HiFid Taq HS Premix (2X)*1	1 ml	1 ml x 5
ROX Reference Dye (50X)*2	40 µl	200 µl

*1 Contains DTI HiFid Taq HS, dNTP Mixture, Mg2+, and Tli RNaseH

*2 Use when performing analyses with real-time PCR instruments that normalize fluorescent signals between wells, such as Applied Biosystems instruments.

- ♦ Add ROX Reference Dye (50X) in a volume equivalent to 1/50 of the PCR reaction mixture when using the following Applied Biosystems systems:
  - 7300 Real-Time PCR System
  - StepOnePlus Real-Time PCR System

- ♦ Add ROX Reference Dye II (50X) in a volume equivalent to 1/100 of the PCR reaction mixture when using the following Applied Biosystems systems:
  - 7500 Real-Time PCR System
  - 7500 Fast Real-Time PCR System
- ♦ No ROX Reference Dye (50X) is required when using any of the following systems:
  - LightCycler/LightCycler 480 System (Roche Diagnostics)
  - CFX96 Real-Time PCR Detection System (Bio-Rad)
  - Smart Cycler System/Smart Cycler II System (Cepheid)

Note: If Rox Reference Dye II is required, Takara Cat# RR390A Premix Ex Taq™ (Probe qPCR) is recommended

4) Storage

Store at 4℃ (stable for up to 6 months).

Every precaution should be taken to avoid contamination.

i. Before use, gently invert tube to make sure reagent is completely dissolved and evenly mixed.

ii. This product may be frozen at -20℃ for long term storage. Once thawed, it should be stored at 4℃ and used within 6 months.

5) Materials required but not provided:

- DNA amplification system for real-time PCR (authorized instruments)
- Reaction tubes or plates designed specifically for the qPCR instrument used
- PCR primers
- Probe for detection
- Sterile purified water
- Micropipette and tips (sterile, with filter)

6) Precautions

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

i. Before use, make sure the reagent is evenly mixed by gently inverting the tube several times without creating bubbles. Uneven reagent mixing will result in inadequate reactivity. Do not mix by vortexing.
When stored frozen at -20℃, DTI HiFid Taq HS Probe Premix (2X conc.) may precipitate. To dissolve the precipitate completely, warm by hand or let stand at room temperature briefly, then invert the tube several times. Make sure reagent is evenly mixed before use.

ii. Place reagent on ice immediately after it has thawed.

iii. This product is not supplied with probe or primers.

iv. Use fresh disposable tips to minimize potential cross-contamination between samples when preparing reaction mixtures or dispensing aliquots.

7) Protocol

i. Protocol

A. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume	Volume	Volume	Final conc.
DTI Probe HiFid Taq HS Premix (2X conc.)	10 µl	12.5 µl	25 µl	1X
PCR Forward Primer (10 µM)	0.4 µl	0.5 µl	1.0 µl	0.2 µM*1
PCR Reverse Primer (10 µM)	0.4 µl	0.5 µl	1.0 µl	0.2 µM*1
Probe*2	0.8 µl	1.0 µl	2.0 µl	
Template*3	2.0 µl	2.0 µl	4.0 µl	
Sterile purified water	6.4 µl	8.5 µl	17 µl	
Total	20 µl*3	25 µl*3	50 µl*3	

*1 Final primer concentration of 0.2 µM is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0 µM.

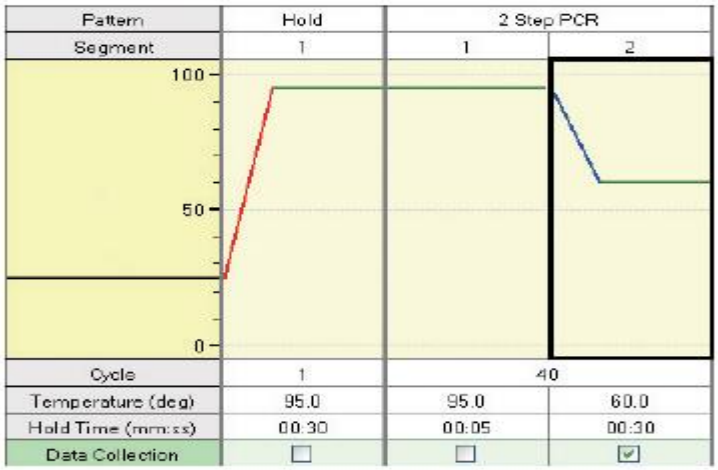
*2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration. When using the Thermal Cycler Dice Real Time System, use a final concentration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

*3 The quantity varies depending on the number of target copies present in the template solution. Make serial dilutions to determine the appropriate template amount and use no more than 100 ng of DNA template.
Furthermore, if cDNA (RT reaction mixture) is used as template, the volume of the RT reaction mixture should be no more than 10% of PCR mixture (e.g., no more than 2.5 µl cDNA template solution for 25 µl PCR reaction).

Note: Depending on the thermal cycler, Use the ROX Reference Dye at a final concentration of 1X or use the ROX Reference Dye II at a final concentration of 0.5X. Adjust the volume of water accordingly in the PCR reaction system

B. Start the reaction.

The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Try this protocol first and optimize PCR conditions as necessary (refer to “PCR Reaction Conditions”)



Shuttle PCR Standard Protocol

Hold (initial denaturation)

Number of cycle: 1

95℃ 30 sec

2-Step PCR

Number of cycles: 40

95℃ 5 sec

60℃ 30 sec

Note:
DTI HiFid Taq HS is a hot-start PCR enzyme that includes an anti-Taq antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95℃ for 30 sec. Longer heat treatment may decrease enzyme activity and affect amplification efficiency and quantification.

C. After the reaction is complete, check the amplification curves and plot a standard curve if absolute quantification will be performed.
Refer to the instrument's instruction manual for specific analysis methods.

ii. PCR Reaction Conditions

Initial denaturation

Step	Temperature	Time	Detection	Comment
Initial denaturation	95℃	30 sec	Off	In general, 95℃ for 30 sec is sufficient for initial denaturation in most cases, even for difficult to denature templates such as circular plasmids and genomic DNA. This procedure may be extended to 1 - 2 min at 95℃ depending on template condition. Prolonged denaturation may inactivate the enzyme. Therefore, do not perform denaturation for more than 2 min.

Shuttle PCR (2-step PCR) number of cycles: 30 - 45 cycles

Step	Temperature	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 ~3 - 5 sec is

				usually sufficient.
Annealing/ extension	56 - 64℃	20 - 30 sec (31, 34 sec)*	On	When optimizing reaction conditions, evaluate results using annealing/ extension temperature in the range of 56 - 64℃. If poor reactivity occurs, increasing incubation time for this step may improve results.

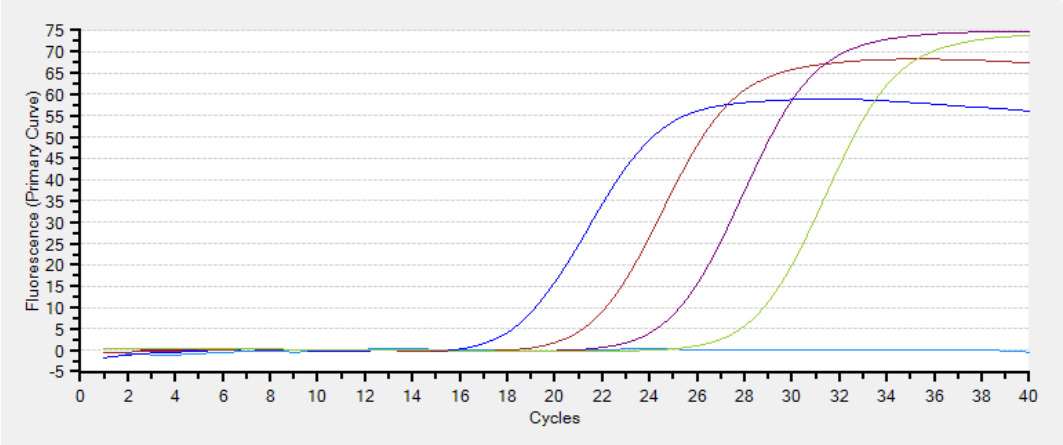
* Some apparatuses do not allow a detection-step setting of 30 sec or shorter.
Applied Biosystems 7300 allows a setting of 31 sec or longer.
Applied Biosystems 7500 allows a setting of 34 sec or longer.

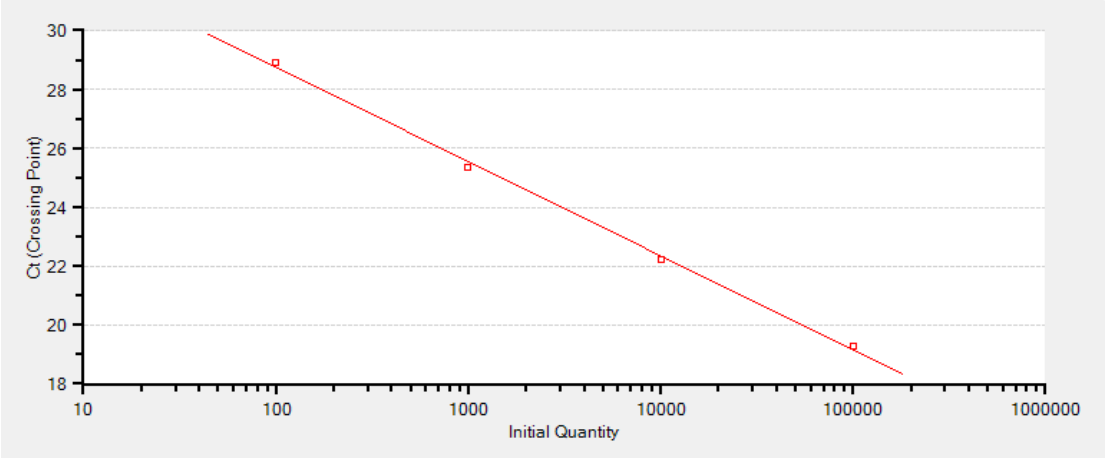
iii. Real-Time RT-PCR:

To synthesize cDNA templates for real-time RT-PCR, we recommend the PrimeScript™ RT Reagent Kit (Perfect Real Time) (Cat. #RR037A). The cDNA is further used as template to perform PCR

iv. Experimental sample:

Consistent amplification was detected by real time PCR using serial dilutions of human testis cDNA from 100 ng to 100 pg for ApoE gene. No amplification was detected in reaction 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.





8) Related Products

Probe qPCR Mix (Cat. #RR391A/B)
PrimeScript™ RT Reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)
One Step PrimeScript™ RT-PCR Kit (Perfect Real Time) (Cat. #RR064A)

Visit https://store.dsstakarabio.com/pages/dti-hifid-taq-hs-probe-premix for more detailed product information

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Email: enquiries@dsstakarabio.com
Toll-Free number 1800-212-4922

Description of Symbol Used:

REF Catalogue number
LOT Batch Code
Date of Manufacturing
Use-by-date
Contains sufficient for <n> tests