DTI Green HiFid Taq HS II premix

Instruction Manual for use

User Manual for DTI Green HiFid Taq HS II premix

Catalogue number: DT0601.100, DT0601.500

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 Green HiFid Taq HS II premix is a reagent specifically designed for intercalator based real-time PCR. The premix is supplied as a convenient, ready-to-use 2X concentrate and contains intercalator dye at a concentration appropriate for real-time monitoring. Simply add primers, template, and sterile purified water to perform intercalator-based real-time PCR. The 2X premix also contains Tli RNaseH, a heat-resistant RNase H, which minimizes PCR inhibition due to residual mRNA when using cDNA as the template.

This product contains a modified buffer with higher reaction specificity than DTI Green HiFid Taq HS premix (Cat. #DT0602). The inhibition of non-specific amplification, which can interfere with quantification, allows accurate measurement over a wide dynamic range. The combination of this buffer and DTI Hifid Taq HS, an efficient hot-start PCR enzyme that uses an anti-Taq antibody, allows highly sensitive, reproducible, and reliable real-time PCR amplification and analysis.

2) Principle

This product includes DTI Hifid Taq HS DNA polymerase for PCR amplification. PCR amplification products may be monitored in real time using the intercalator dye used in the product.

i. PCR

PCR is a technique used to amplify a target sequence from a minute amount of DNA. By repeating cycles of denaturation, primer annealing, and elongation, the target gene fragment may be quickly amplified using DNA polymerase.
This product uses DTI Hifid Taq HS, a hot-start PCR enzyme that prevents non-specific amplification from mispriming or primer-dimer formation during reaction mixture preparation or other pre-cycling steps, allowing highly sensitive detection.

ii. Fluorescence Detection - Intercalator Method

This method involves the addition of an intercalating agent that fluoresces when bound to double-stranded DNA in the reaction mixture.
Monitoring this fluorescence enables the detection of amplified DNA, quantitative determination of target DNA, and determination of DNA composition by melting curve analysis.

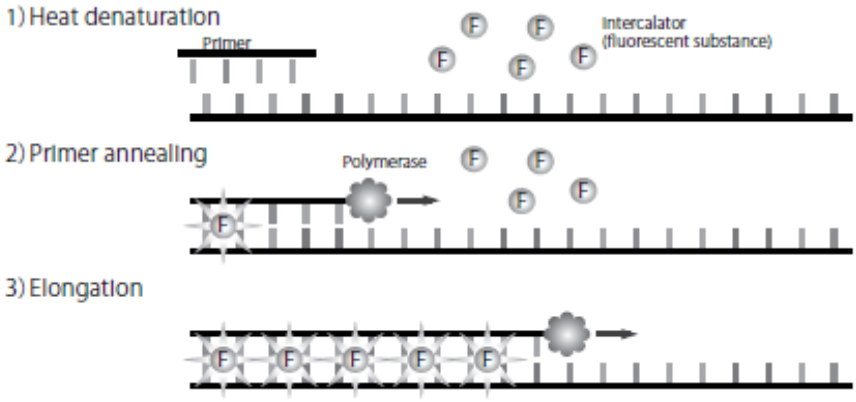


Figure 1. Fluorescent intercalator detection method.

3) Components (for 20 µl per reaction)

Components	Cat# DT0601.100 (100 rxn)	Cat# DT0601.500 (500 rxn)
DTI Green HiFid Taq HS II premix (2X)*1	1 ml	1 ml x 5
ROX Reference Dye (50X)*2	40 µl	200 µl

*1 Contains DTI Hifid Taq HS DNA polymerase, dNTP mixture, Mg2+, Tli RNaseH, and an intercalator dye.

*2 ROX Reference Dye are intended for use with instruments that correct for between-well fluorescent signal, such as the real-time PCR devices by Applied Biosystems.

- ♦ Use ROX Reference Dye:
  - Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific)
  - Applied Biosystems Step OnePlus Real-Time PCR System (Thermo Fisher Scientific)
- ♦ Use ROX Reference Dye II:
  - Applied Biosystems 7500/7500 Fast Real-Time PCR System (Thermo Fisher Scientific)
- ♦ Do not use reference dye:
  - LightCycler/LightCycler 480 System (Roche Diagnostics)
  - CFX96 Real-Time PCR Detection System (Bio-Rad)

Note: If Rox Reference Dye II is required, Takara Cat# RR820A TB Green Premix Ex Taq II (Tli RNaseH Plus) is recommended.

4) Storage

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

Always protect from light and avoid contamination.

For long-term storage, store at -20°C. Store thawed or opened product at 4°C and use within 6 months.

5) Materials required but not provided:

i. Reagents

- PCR primers
  Guidelines for real-time PCR primer design are found in Section 9.1
- Sterile purified water

ii. Materials

- Real-time PCR reaction tubes or plates designed specifically for the qPCR instrument used
- Micropipettes and tips (sterile filter tips)
- Gene amplification system for real-time PCR (authorized instruments)
  Compatible instruments include:
  - Applied Biosystems 7300, 7500, or 7500 Fast Real-Time PCR Systems, StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
  - LightCycler/LightCycler 480 System (Roche Diagnostics)
  - CFX96 Real-Time PCR Detection System (Bio-Rad)

Note: DTI Green HiFid Taq HS premix (Cat. #DT0601) is recommended when using the Smart Cycler System/Smart Cycler II System (Cepheid).

6) Precautions

Read these precautions before use and follow them carefully.

i. Prior to use, make sure the reagent is evenly mixed by gently inverting several times without creating bubbles. Uneven reagent mixture will result in inadequate reactivity.
   a. Do not mix by vortexing.
   b. When stored at -20°C, DTI Green HiFid Taq HS II premix may develop a white to pale yellow precipitate. Gently hand-warm and allow to stand protected from light at room temperature briefly, then invert several times to dissolve the precipitate completely.
   c. The presence of precipitate is indicative of uneven reagent composition; make sure the reagent is evenly mixed before use.

ii. Place reagents on ice immediately after thawing, and them keep on ice while preparing the reaction mixture.

iii. This product contains an intercalator dye. Avoid exposure to bright light while preparing the reaction mixture.

iv. While preparing or dispensing reaction mixtures, use sterile, disposable tips to avoid contamination between samples.

7) Protocol

A. Prepare the PCR mixture shown below. To account for pipetting error, make at a master mix with least 10% more than the total volume needed for the total number of reactions.
<Per reaction>

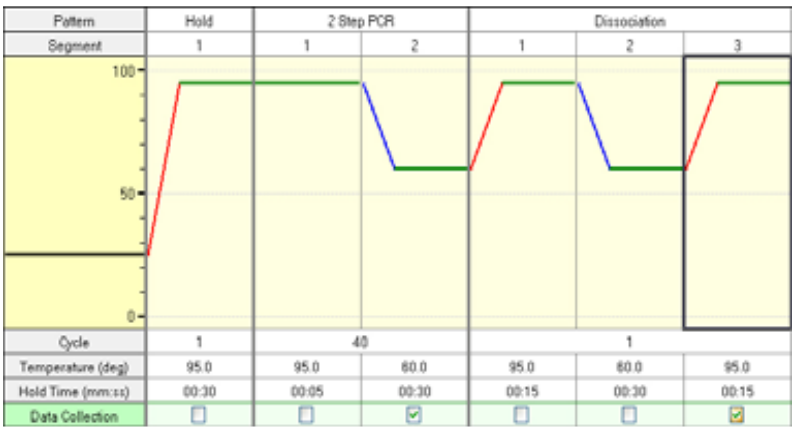
Reagent	Volume	Volume	Volume	Final conc.
DTI Green HiFid Taq HS II premix (2X)	10 µl	12.5 µl	25 µl	1X
PCR Forward Primer (10 µM)	0.8 µl	1.0 µl	2.0 µl	0.4 µM*1
PCR Reverse Primer (10 µM)	0.8 µl	1.0 µl	2.0 µl	0.4 µM*1
Template (<100 ng)*2	2.0 µl	2.0 µl	4.0 µl	
Sterile purified water	6.4 µl	8.5 µl	17 µl	
Total	20 µl	25 µl	50 µl	

*1 A final primer concentration of 0.4 µM is likely to yield good results.
However, if there is insufficient reactivity, use a primer concentration between 0.1 and 1.0 µM.

*2 The optimal 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 per 25 µl.
Furthermore, if cDNA (RT reaction mixture) is used as the template, the volume of the RT reaction mixture should not exceed 10% of the PCR mixture.

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 shuttle PCR standard protocol is recommended; try this protocol first and optimize PCR conditions as necessary. Perform a 3-step PCR when using primers with low Tm values or when shuttle PCR is not feasible. To optimize PCR conditions, please refer to Section 8).



Hold (Initial Denaturation)
Cycle: 1
95°C 30 sec
2-Step PCR
Cycles: 40
95°C 5 sec
60°C 30 sec
Dissociation

Figure 2. Shuttle PCR standard protocol.

Note:
DTI Hifid Taq HS DNA polymerase is a hot-start PCR enzyme with an anti-Taq antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95°C 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 and melting curves and plot a standard curve if quantification will be performed.
Refer to the instrument's instruction manual for specific analysis methods.

8) Optimization

If the recommended conditions (shuttle PCR standard protocol) do not provide sufficient reactivity, follow the procedures below to optimize primer concentration and PCR conditions. Depending on the reaction system, switching to a different real-time PCR reagent from the Perfect Real Time series (Cat. #DT0602,) may greatly improve the results.

Select PCR conditions based on comprehensive analysis of reaction specificity and amplification efficiency. Using conditions that balance these two aspects will allow accurate measurement over a wide range of concentrations.

- System with a high reaction specificity
  - Using a negative, no-template control, non-specific amplification (e.g., primer dimers) does not occur.
  - Non-specific amplification products (those other than the target product) are not generated.

- System with a high amplification efficiency
  - Amplification product is detected at earlier cycles (lower Ct value).
  - PCR amplification efficiency is high (near the theoretical value of 100%).

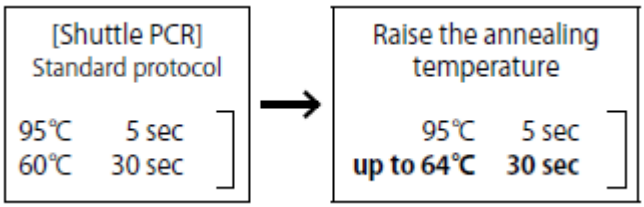
i. Evaluation of primer concentration

The relationship between primer concentration, reaction specificity, and amplification efficiency is illustrated below. Reducing primer concentration raises reaction specificity whereas increasing the primer concentration raises amplification efficiency.

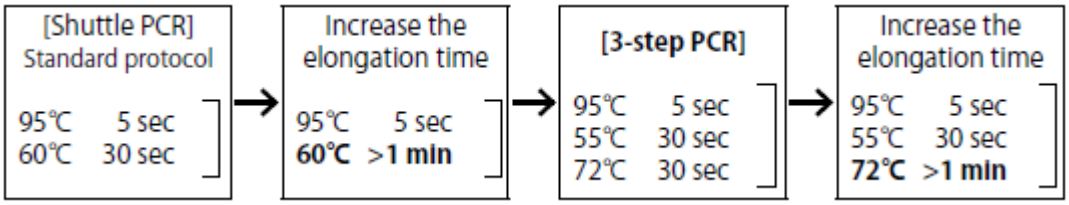
Primer concentration) Low (0.2 µM) High (1.0 µM)
Specificity High ← Low
Efficiency Low → High

ii. Evaluation of PCR conditions

- To improve reaction specificity
  Raising the annealing temperature may improve reaction specificity. Perform optimization while checking amplification efficiency.



- To improve amplification efficiency
  Increasing the elongation time or switching to 3-step PCR may improve amplification efficiency. Perform optimization using the steps below.



- Initial denaturation
  Generally, 95℃ for 30 sec is sufficient for initial denaturation, 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 the template. However, prolonging this step may inactivate the enzyme. Therefore, it is recommended to avoid initial denaturation steps >2 min.

iii. Relationship between reagent and reactivity

DTI supplies several different reagents for intercalator-based real-time PCR analysis using TB Green. The relationship between reaction specificity and amplification efficiency for these reagents is described below.

DTI Green HiFid Taq HS premix (Cat. #DT0602) DTI Green HiFid Taq HS II premix (Cat. #DT0601) have greater specificity.

(Reagent)	DTI Green HiFid Taq HS premix	DTI Green HiFid Taq HS II premix
Specificity	Lower ←	→ Higher
Efficiency	Higher →	Lower

9) Appendix

i. Primer design

Designing primers with good reactivity is critical to successful real-time PCR. Follow the guidelines below to design primers that yield high amplification efficiency without non-specific amplification.

RT-PCR primers designed and synthesized using these guidelines are compatible with the shuttle PCR standard protocol (Section 7).

- Amplification Product

Amplification size	The optimal size is 80 - 150 bp (amplification up to 300 bp is possible).

- Primer

Length	17 - 25 mer
GC content	40 - 60% (preferably 45 - 55%)
Tm	Make sure the Tm values for the forward primer and the reverse primer do not differ greatly. Use software to determine Tm values. OLIGO*1 : 63 - 68°C Primer3 : 60 - 65°C
Sequence	Make sure there are no overall base sequence biases. Avoid having any GC-rich or AT-rich regions in the sequence (especially at the 3' end). Avoid having consecutive T/C pairings (polypyrimidine). Avoid having consecutive A/G pairings (polypurine).
3' end sequence	Avoid having GC-rich or AT-rich regions at the 3' end. It is preferable to have a G or C as the terminal base at the 3' end. Avoid a primer design with T as the terminal base at the 3' end.
Complementation	Avoid having any complementary sequences of three bases or more within a primer and between primers. Avoid having any complementary sequences of two bases or more at a primer's 3' end.
Specificity	Verify primer specificity using a BLAST search *2

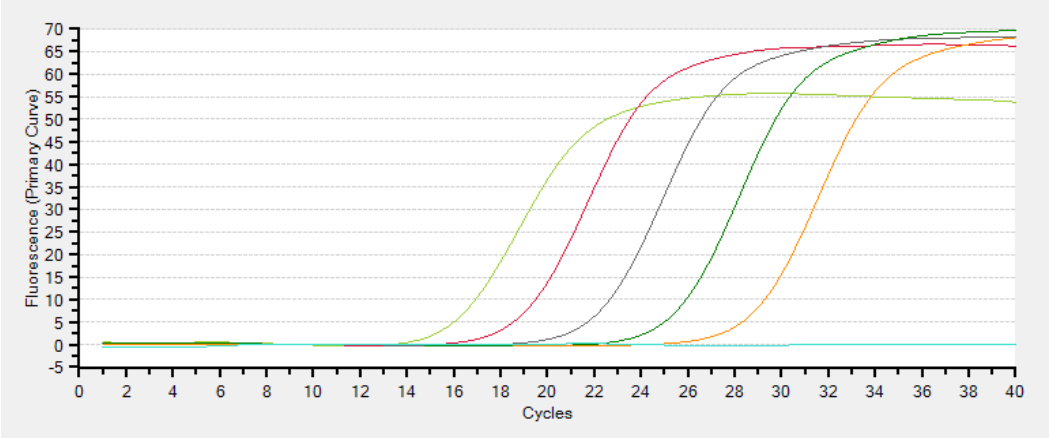
*1 OLIGO Primer Analysis Software (Molecular Biology Insights, Inc.)
*2 https://blast.ncbi.nlm.nih.gov/Blast.cgi

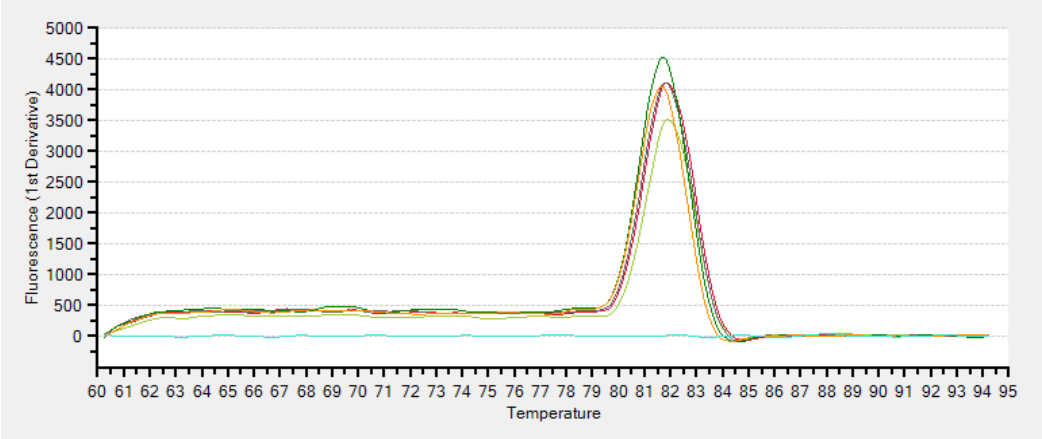
ii. Preparing templates for real-time RT-PCR

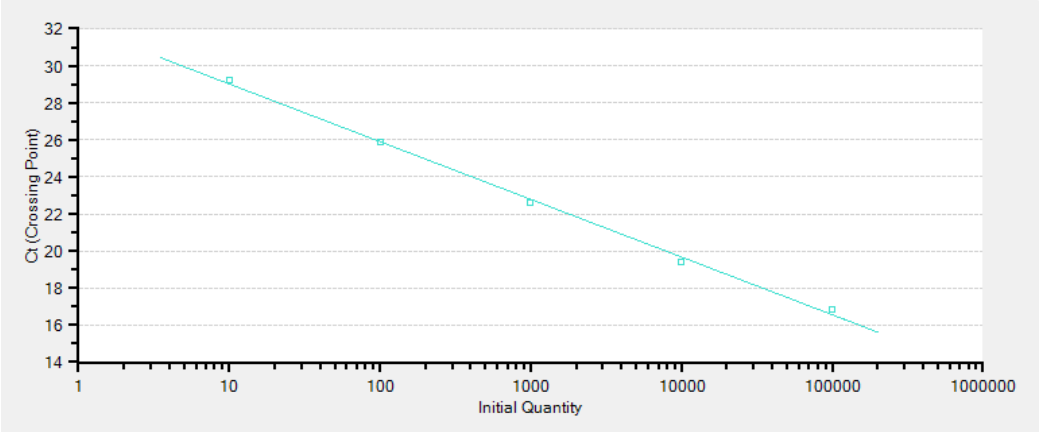
To synthesize cDNA templates for real-time RT-PCR, we recommend the PrimeScript™ RT Reagent Kit (Perfect Real Time) (Cat. #RR037A). The cDNA is further used as template to perform PCR

10) Experimental sample:

Consistent amplification was detected by real time PCR using serial dilutions of human cDNA from 100 ng to 10 pg for H32 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, melting curve and standard curve.







11) Related Products

DTI Green HiFid Taq HS Premix (Cat. #DT0602)
TB Green® Premix Ex Taq ™ GC (Perfect Real Time) (Cat. #RR071A/B)
PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)
PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A/B)

Visit https://store.dsstakarabio.com/pages/dti-green-hifid-taq-hs-ii-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