DTI Green HiFid Taq HS premix

Instruction Manual for use

User Manual for DTI Green HiFid Taq HS premix

Catalogue number: DT0602.100, DT0602.500

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 premix is designed for intercalator-based real-time PCR. It is supplied at a 2X concentration and includes an intercalator dye at a concentration appropriate for real time monitoring, making it easy to prepare reaction mixtures. The 2X premixed reagent also contains Tli RNase H, a heat-resistant RNase H that minimizes PCR inhibition by degrading residual mRNA when using cDNA as template.

The combination of DTI Hifid Taq HS, a hot-start PCR enzyme that includes an anti-Taq antibody, and a buffer optimized for real-time PCR, allows high amplification efficiency and detection sensitivity. This product is suitable for high-speed PCR and enables accurate assay and detection of targets, making it possible to obtain highly reproducible and reliable real-time PCR results.

Benefits

i. Allows rapid and accurate detection and assay of targets by real-time PCR.
ii. 2X concentration premixed with intercalator dye; simply add primers, template, and sterile purified water to perform intercalator-based real-time PCR.
iii. DTI Hifid Taq HS, a hot-start PCR enzyme, is used for PCR. The buffer system has been optimized for real-time PCR, allowing good amplification efficiency and high-sensitivity detection.
iv. The 2X reagent is premixed with Tli RNaseH, a heat-resistant RNase H that minimizes PCR inhibition by residual mRNA when using cDNA as template.

2) Principle

This product uses DTI Hifid Taq HS for PCR amplification. PCR amplification products may be monitored in real time using an intercalator dye.

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 non-specific 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 - Intercalator Method

This method uses a DNA intercalator dye that emits fluorescence when bound to double-strand DNA. Monitoring fluorescence allows for quantification of amplification products.

Measuring the fluorescence intensity also provides the melting temperature of amplified DNA.

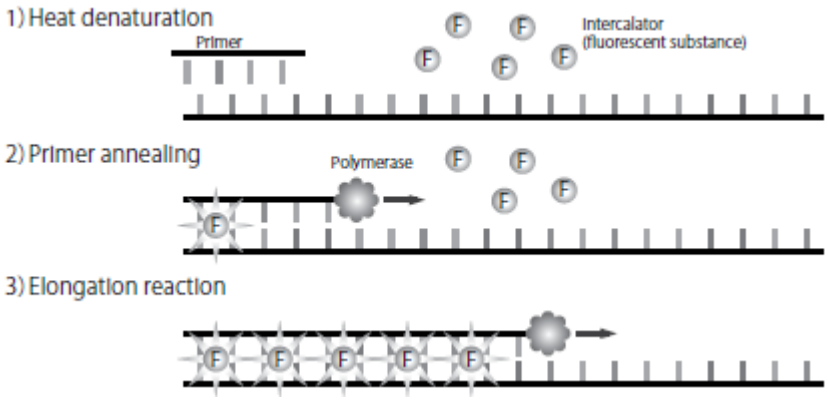


Figure 1. Fluorescent intercalator detection method.

3) Components (20 µl per reaction)

Components	Cat# DT0602.100 (100 rxn)	Cat# DT0602.500 (500 rxn)
DTI Green 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+, Tli RNase H, and and an intercalator dye.
*2 ROX Reference Dye is used for analyses with devices 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 StepOnePlus 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 this component
  - Smart Cycler II System (Cepheid)
  - LightCycler/LightCycler 480 System (Roche Diagnostics)
  - CFX96 Real-Time PCR Detection System (Bio-Rad)

Note: If Rox Reference Dye II is required, Takara Cat# RR420A TB Green Premix Ex Taq (Tli RNase H 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
- Micropipettes and tips (sterile filter tips)
- Gene amplification system for real-time PCR (authorized instruments)
  Compatible instruments include:
  - Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System, StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
  - LightCycler/LightCycler 480 System (Roche Diagnostics)
  - Smart Cycler II System (Cepheid)
  - CFX96 Real-Time PCR Detection System (Bio-Rad)

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

ii. Place reagents on ice when preparing the reaction mixture.

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

iv. Use fresh disposable tips to avoid contamination between samples when preparing or dispensing reaction mixtures.

7) Protocol

A. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume	Volume	Volume	Final conc.
DTI Green HiFid Taq HS premix (2X)	10 µl	12.5 µl	25 µl	1X
PCR Forward Primer (10 µM)	0.4 µl	0.5 µl	1 µl	0.2 µM*1
PCR Reverse Primer (10 µM)	0.4 µl	0.5 µl	1 µl	0.2 µM*1
Template (<100 ng)*2	2.0 µl	2.0 µl	4 µl	
Sterile purified water	7.2 µl	9.5 µl	19 µl	
Total	20 µl	25 µl	50 µl	

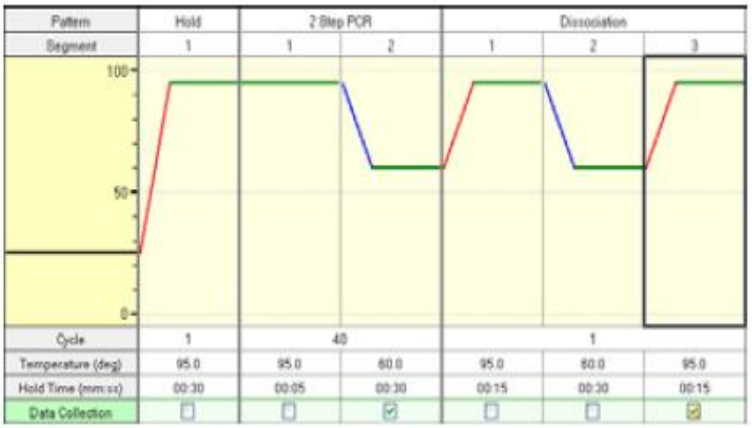
*1 A final primer concentration of 0.2 µM likely will provide good results. However, if there is an issue with reactivity, use a primer concentration between 0.1 and 1.0 µM.

*2 The optimal amount varies depending on the number of target copies present in thetemplate solution. Make serial dilutions to determine the appropriate amount and use no more than 100 ng of DNA template. 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.

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. Use this protocol first and optimize PCR conditions when necessary. Perform 3-step PCR when using primers with low Tm values or when a shuttle PCR is not feasible. (For optimizing 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 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°C for 30 sec. Longer heat treatment may decrease enzyme activity and affect amplification efficiency and quantification.
- For initial template denaturation before PCR, 95°C for 30 sec is sufficient.

C. After the reaction is complete, check the amplification and melting curves and plot a standard curve if absolute quantification will be performed. When using Thermal Cycler Dice Real Time System, please refer to the instruction manual for analysis methods.

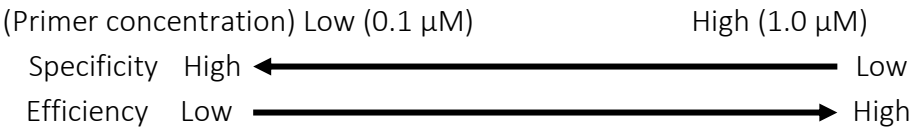
8) Optimization

If unsatisfactory results are obtained using the recommended conditions (shuttle PCR standard protocol), follow the procedures below to optimize the primer concentration and PCR conditions. In addition, depending on the reaction system, switching to another Premix (Cat. #DT0601) may greatly improve the results. Select PCR conditions based on comprehensive analysis of both 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
  - With 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 early (small Ct value).
  - PCR amplification efficiency is high (near the theoretical value of 100%).

i. Evaluation of primer concentration

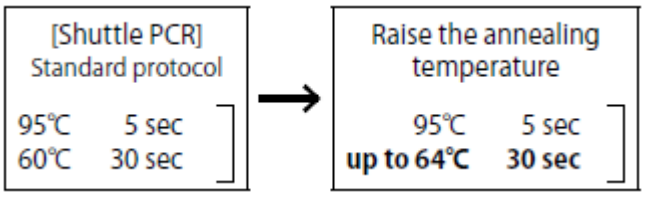
The relationship between primer concentration and reaction specificity and amplification efficiency is illustrated below. Reducing primer concentration raises reaction specificity. In contrast, increasing primer concentration raises amplification efficiency.



ii. Evaluation of PCR conditions

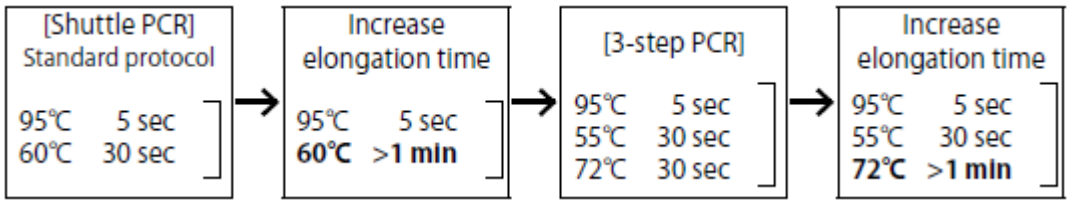
- To improve reaction specificity

  Raising the annealing temperature may improve reaction specificity. Perform optimization while checking for effects on 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°C for 30 sec is sufficient for initial denaturation, even for difficult-to denature templates such as circular plasmids and genomic DNA. This step may be extended to 1 - 2 min at 95°C depending on the template. However, prolonging this step may inactivate the enzyme. Therefore, it is recommended to avoid initial denaturation steps longer than 2 min.

iii. Relationship between reagent and reactivity

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

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

(Reagent)

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

9) Appendix

i. Primer design

Designing primers with good reactivity is critical to efficient real-time PCR. Please 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 standard shuttle PCR protocol (Section VII.).

- 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 primer design software to determine Tm values. OLIGO*1 : 63 - 68°C Primer3 : 60 - 65°C
Sequence	Make sure that overall there are no base sequence biases. Avoid having any GC-rich or AT-rich regions in the sequence (particularly at the 3' end). Avoid having consecutive T/C pairings (polypyrimidine). Avoid having consecutive A/G pairings (polypurine).
3' end sequence	Avoid having any GC-rich or AT-rich sequence at the 3' end. It is preferable to have a G or C as the 3' end-base. Avoid primers with T as the 3' end-base.
Complementation	Avoid having any complementary sequences of 3 bases or more within a primer and between primers. Avoid having any complementary sequences of 2 bases or more at the primer's 3' ends.
Specificity	Verify primer specificity using a BLAST search *2

*1 OLIGO Primer Analysis Software (Molecular Biology Insights, Inc.)

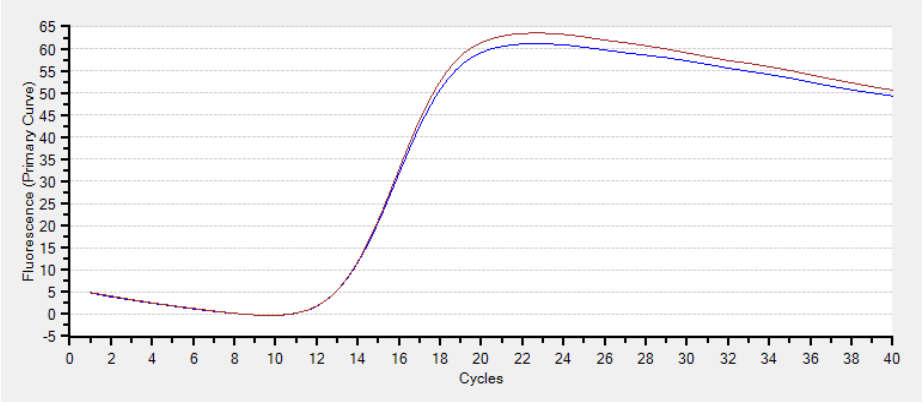
*2 https://blast.ncbi.nlm.nih.gov/Blast.cgi

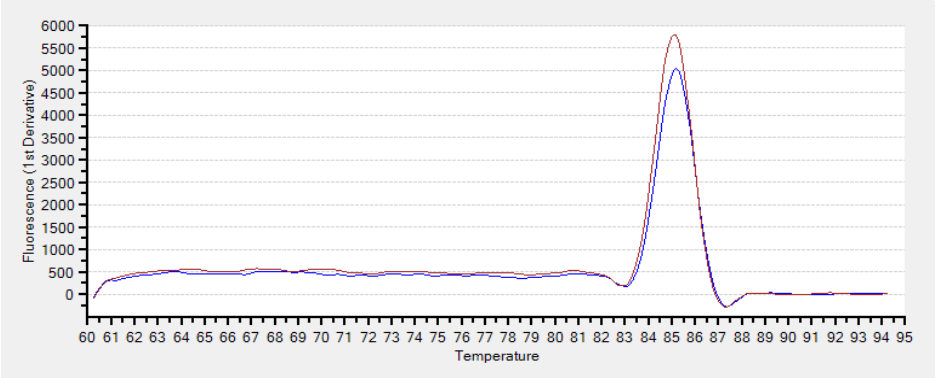
ii. Preparing templates for real-time RT-PCR

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 of DNA was detected by the fluorescence of intercalator dye in real-time PCR using λ DNA as a template. No specific amplification was observed without template. Below are the images of amplification plots and melting curve.





11) Related Products

DTI Green HiFid Taq HS premix II (Cat. #DT0601.100/DT0601.500)
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)

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

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