# DTI FabTaq HS-Glycerol free (50U/ul)

Instruction Manual for use

User Manual for DTI FabTaq HS-Glycerol free (50U/ul)
For further manufacturing use

Catalogue number: DT0103.2500

Manufactured by
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## 1) Product Description:

DTI FabTaq HS-Glycerol free (50 U/ul) is a hot start PCR enzyme derived from Thermus aquaticus that includes a neutralizing monoclonal antibody that recognizes Taq DNA polymerase. This antibody binds to Taq DNA polymerase and prevents non-specific amplification due to mispriming and/or formation of primer dimers before thermal cycling. The antibody is denatured during the initial DNA-denaturation step, allowing this product to be used with standard PCR conditions. Since the product is glycerol free, it is suitable for lyophilization processes

## 2) Kit Contents:

| Component | Qty |
|---|---|
| DTI FabTaq HS-Glycerol free (50 U/ul) | 50 µl |

* Customized pack size available

## 3) Storage and shipment conditions: -80°C

## 4) Materials required but not provided:

10X PCR buffer

25 mM/50 mM MgCl2

2.5 mM dNTP

Template DNA/cDNA

Target specific forward and reverse primers

Nuclease free water

Thermal cycler

Agarose electrophoresis system

Loading dyes and marker

## 5) Unit definition:

One unit is the amount of enzyme that will incorporate 10 nmol of dNTPs into acid-insoluble products in 30 minutes at 74°C with activated salmon sperm DNA as the template-primer.

## 6) Reaction mixture for unit definition :

| | |
|---|---|
| 25 mM | TAPS (pH 9.3 at 25℃) |
| 50 mM | KCl |

| | |
|---|---|
| 2 mM | MgCl2 |
| 0.1 mM | DTT |
| 200 µM | each dATP・dGTP・dCTP |
| 100 µM | [3H]-dTTP |
| 0.25 mg/ml | activated salmon sperm DNA |

## 7) Purity :

Nicking, endonuclease, and exonuclease activity were not detected after incubation of 0.6 µg of supercoiled pBR322 DNA, 0.6 µg of λDNA, or 0.6 µg of λ-Hin d III digest with 10 U of this enzyme for 1 hour at 74°C.

## 8) Application :

- For DNA amplification by hot start PCR
- For DNA sequencing
- For further manufacturing and lyophilization processes

## 9) PCR products :

As most PCR products amplified with Taq HS have one A at the 3'-termini, the obtained PCR products can be directly cloned into a T-vector. It is also possible to clone the product in blunt-end vectors after blunting and phosphorylation of the ends.

## 10) General reaction mixture

### a) For endpoint PCR (total 50 µl)

As per the requirement, use the FabTaq HS-Glycerol free (50U/ul) as is. If needed, the enzyme could be diluted, using the dilution buffer (not provided in the kit)a

| | |
|---|---|
| FabTaq HS (5 U/µl) | 0.25 µl |
| 10X PCR Buffer (Mg2+ free) | 5 µl |
| 50 mM MgCl2 | 1.5 ul |
| dNTP Mixture (2.5 mM each) | 4 µl |
| Template | < 500 ng |
| Primer 1 | 10 - 50 pmol (final conc. 0.2 - 1.0 µM) |
| Primer 2 | 10 - 50 pmol (final conc. 0.2 - 1.0 µM) |
| Sterile purified water | up to 50 µl |

Note:

Reaction mixtures can be set up at room temperature. Be sure to keep all reagents on ice.

PCR conditions :

This enzyme can be used with standard PCR conditions, since the monoclonal antibody is denatured in the initial DNA-denaturation step. There is no need for an additional step to denature the anti-Taq antibody.

Example : Amplification of a 1 kb DNA fragment

| | | |
|---|---|---|
| 98℃ | 10 sec | 30 cycles |
| 55℃ | 30 sec | |
| 72℃ | 1 min | |

Note:

i. Denaturation conditions vary depending on the thermal cycler and tubes used for PCR. Denaturation for 5 - 10 sec at 98℃, or 20 - 30 sec at 94℃ is recommended.

ii. The annealing step should be adjusted for each primer set; the annealing temperature depends directly on the Tm of primers. Typically, the annealing temperature is set to be 3 ~ 5°C lower than the Tm value of the primers.

b) For real time PCR (total 25 µl)

As per the requirement, use the FabTaq HS-Glycerol free (50U/ul) as is. If needed, the enzyme could be diluted, using the dilution buffer provided in the kit

| | |
|---|---|
| 10X PCR Buffer (Mg2+ free) | 2.5 µl |
| 50 mM MgCl2 | 0.75 ul |
| FabTaq HS (5 U/µl) | 0.25 µl |
| dNTP Mixture (2.5 mM each) | 2 µl |
| Forward Primer (10 µM)* | 0.5 µl |
| Reverse Primer (10 µM)* | 0.5 µl |
| Probe (10 µM)** | 0.5 µl |
| Template*** | 2 µl |
| Sterile purified water | up to 25 µl |

* A final primer concentration of 0.2 µM is most likely to yield good results. However, should further optimization be required, try adjusting primer concentrations in the range of 0.1 to 1.0 µM.

** 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 to 0.5 µM.

*** The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2 µl cDNA template solution for a 20 µl PCR reaction).

**Note:**

Reaction mixtures can be set up at room temperature. Be sure to keep all reagents on ice.

**PCR conditions :**

| | | |
|---|---|---|
| 94 C | 30 sec | |
| 95 C | 5 sec | 40 Cycles |
| 60 C | 15 sec* | |

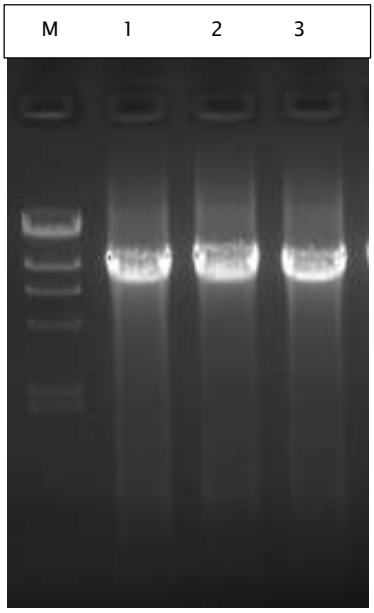
*Collection of fluorescence signal

**11) Quality control data:** Please see the certificate of analysis for each lot

**13) Experimental sample:**

a) Amplification data for end point PCR: Good performance of this product has been confirmed by PCR using λ DNA as template (amplified fragment size 8 kbp). The protocol used for the assay and the results are as follows:

| | | |
|---|---|---|
| 94C | 1 min | |
| 94C | 30 sec | 30 cycles |
| 65C | 10 min | |
| 4C | ∞ | |




Lane M: λ Hind III digest marker
Lane 1-3: Test in triplicates

b) Hot start function data:

Test target sample added to a PCR system containing FabTaq and FabTaq HS respectively. Following an incubation at RT for 30 mins, the products were amplified using a thermal cycler. Non specific bands (around 280 bp) were observed in the PCR system along with specific target bands (838 bp) using FabTaq, while there were no non specific bands observed in PCR system using FabTaq HS.

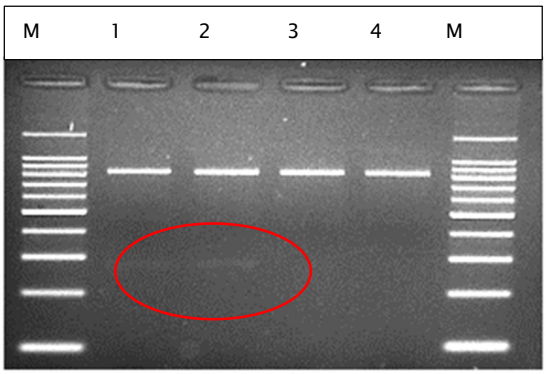
The protocol used for the assay and the results are as follows:

Incubate at 25C for 30 mins

↓

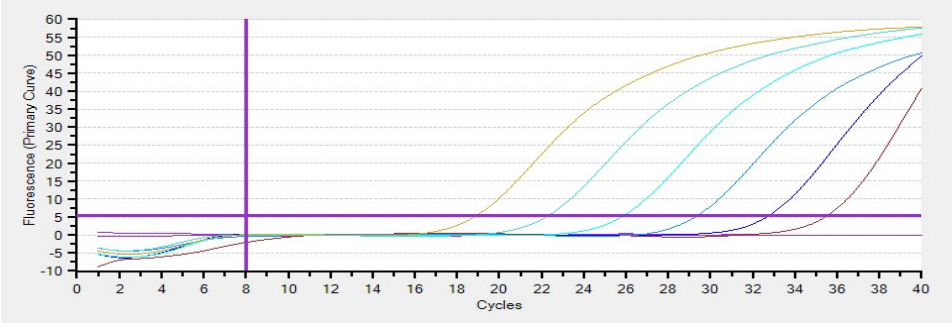
Place the tubes in thermal cycler and run the program

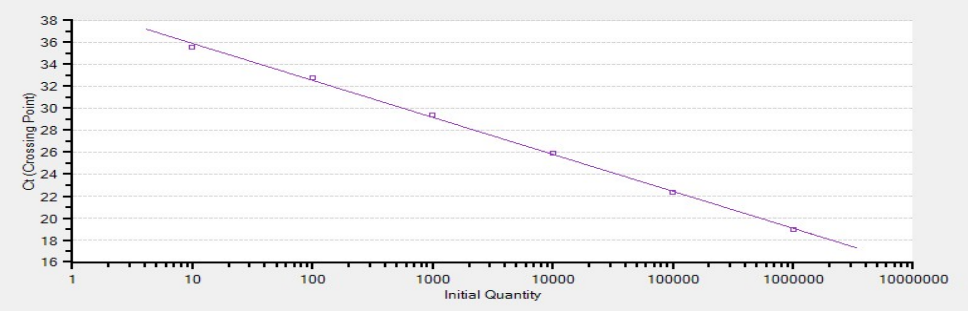
| | | |
|---|---|---|
| 94C | 30 sec | 25Cycles |
| 55C | 30 sec | |
| 72C | 30 sec | |
| 72C | 5 min | |




Lane M: 100 bp DNA marker
Lane 1-3: FabTaq PCR system
Lane 4-6: FabTaq HS PCR system

c) qPCR amplification data: 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.




Visit https://store.dsstakarabio.com/ 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