DTI Bca DNA Polymerase- Glycerol Free (8U/µl)

Instruction Manual for use

User Manual for DTI Bca DNA Polymerase- Glycerol Free (8U/µl)

Catalogue number: DT0104.80

1) Product Description:

DTI Bca DNA Polymerase- Glycerol Free originates from a thermophilic bacterium Bacillus caldotenax, that lacks 5' → 3' exonuclease activity. By introducing mutations into the protein, the activity of both DNA polymerase and reverse transcriptase is highly enhanced while exerting the original strong strand-displacement activity. Therefore, the isothermal amplifications using this enzyme enable to amplify the target product from a little nucleic acid in a shorter time. This enzyme is originally isolated and developed independently by Takara Bio Inc, Japan. DTI manufactures and sells it under an agreement with Takara Bio Inc.

2) Kit Contents:

Component	Qty
DTI Bca DNA Polymerase- Glycerol Free (8U/µl)	10 µl

* Customized pack size available

3) Storage and shipment conditions: -80°C

4) Materials required but not provided:

2X BcaBEST buffer (Component of Takara Cat# RR380A)

10X target specific LAMP primer mix

Template DNA/cDNA

For RT-LAMP, Reverse Transcriptase

Nuclease free water

for real time LAMP assay: Inter-calating dye , Thermal cycler

5) Source:

Escherichia coli carrying a plasmid containing the gene for Bca DNA Polymerase.

6) Properties:

Molecular Mass: approx. 68 kDa

7) Unit definition:

One unit is defined as the amount of enzyme that incorporates 210 nmol of dNTP into acid-insoluble precipitate in 30 minute at 65°C.

8) Application :

1. LAMP (Loop-Mediated Isothermal Amplification)

2. RCA (Rolling Circle Amplification)

9) Precautions for use:

1. Don't mix the enzyme vigorously.
2. Extreme cautions should be taken during reaction preparation to avoid contamination. If analysing the products after reaction, execute in a different area from the reaction preparation.
3. Reaction temperatures above 70℃ are not recommended. Hence this enzyme can't be used for conventional PCR.

10) Usage protocol

LAMP Reaction Example:

Nuclease-free water	x µl
2X BcaBEST Buffer	12.5 µl
10X LAMP primer mix*1	2.5 µl
DTI Bca DNA Polymerase-Glycerol Free (8U/ul)	0.3 - 1 µl*2
Sample*3	e.g., 2 µl
Total	**25 µl**

Incubate at 60 - 65°C for 30 minutes.*4

*1 FIP/BIP primers : 16 µM
F3/B3 primers : 2 µM
LoopF/B primers : 8 µM

*1,2,4 Optimize reaction systems, including primer and enzyme concentrations, and reaction temperature and time, due to their variations depending on the target and primer sequence. Use of high concentration of enzyme would reduce the detection (amplification) time but might also increase the risk of non-specific amplification. It is strongly recommended to include a no-template control reaction to ensure the specificity.

*3 In order to prevent the non-specific reaction during its preparation, sample should be added lastly. In case of RNA target, add the Reverse Transcriptase XL (AMV) (5 U/µl) to be a final concentration of 0.04 U/µl.

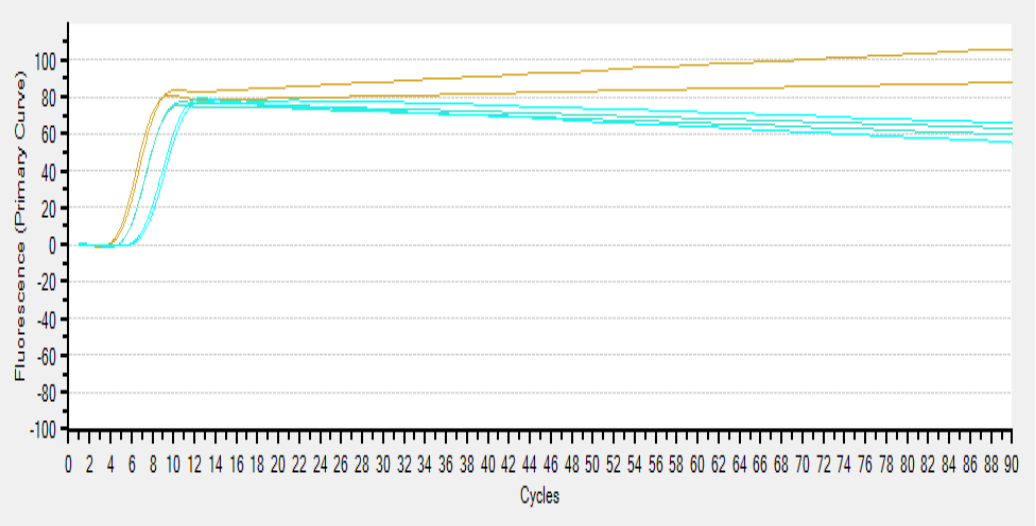
Note: i) 2X BcaBEST Buffer mentioned in the protocol is a component of Takara brand cat# RR380A BcaBEST™ DNA Polymerase ver.2.0

ii) Various detection methods can be combined with the reaction, such as a color indicator detection by eye and a fluorometric detection by real-time instrument. Use an appropriate method depending on your purpose.

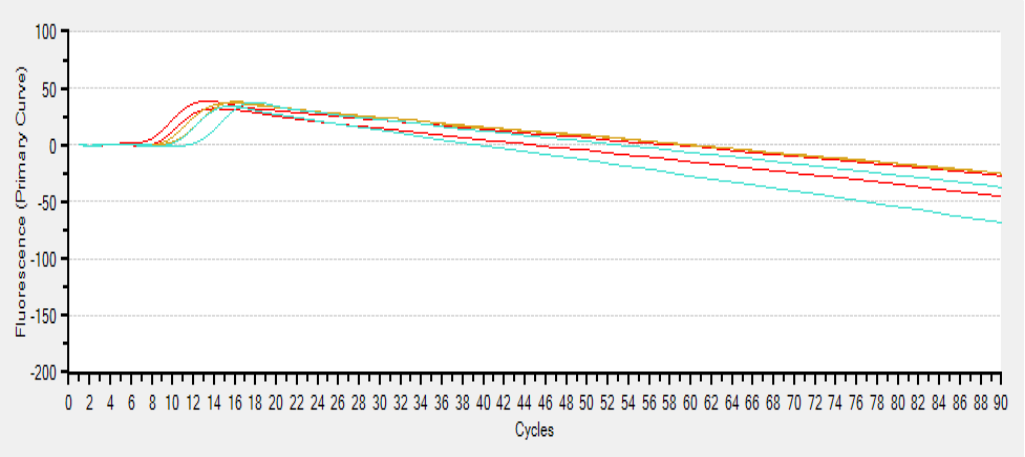
11) **Quality control data:** Please see the certificate of analysis for each lot.

12) **Experimental sample:**

i) LAMP assay: Target gene was detected using 0.01 – 0.01 ng of E. coli genomic DNA when the isothermal amplification was performed using the enzyme. The protocol used was : Incubate at 63℃ for 90 minutes



ii) RT-LAMP assay: Target gene was detected using 0.01 – 0.01 ng of human total RNA when the RT- isothermal amplification was performed using AMV reverse transcriptase (Takara cat# 2630A) and Bca DNA polymerase. The protocol used was: Incubate at 63℃ for 90 minutes



13) Reference:

Uemori,T.; Ishino, Y.; et al. Cloning of the DNA polymerase gene of Bacillus caldotenax and characterization of the gene product. J. Biochem. 1993, Mar;113(3):401-10

Visit https://dsstakarabio.com/pages/dti-bca-dna-polymerase-glycerol-free-8u for more detailed product information.

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Description of Symbol Used:

Catalogue number

Batch Code


Date of Manufacturing


Use-by-date


Contains sufficient for <n> tests