Bst II DNA Polymerase

Please read the manual carefully before use.
Cat. No. LP301
Version. No. Version 2.0
Storage: at -20°C for two years

Description

This product contains *Bst* II DNA polymerase, 5×LAMP Reaction Mix, fluorescent dye TS LAMP Green. It is suitable for LAMP reaction with DNA as template, featuring strong amplification ability and high specificity, and has excellent reaction performance in LAMP reactionusing fluorescence quantitative method (dye method, probe method).

Bst II DNA Polymerase is a recombinant Bacillus stearothermophilus DNA polymerase, which is obtained by purification and isolation after expression in E. coli. The enzyme has 5'→3' DNA polymerase activity and lacks 5'→3' exonucleaseactivity.

5×LAMP Reaction Mix is an optimized reaction mastermix, which already contains $MgSO_4$, dNTPs and other components required for the reaction, saving the time of addition.

TS LAMP Green is a DNA-binding dye for fluorescence quantitative amplification. It has a similar spectrumto SYBR Green I and is compatible with all well-known brands of qPCR instruments. Replacing SYBR Green I with TS LAMP Green does not require any changes to your current procedures and equipment.

Highlights

- Loop-mediated Isothermal Amplification, LAMP
- Fast polymerization
- Strongstrand-displacement capability

Application

- DNA isothermal amplification
- DNA sequencingthroughhigh GCregions
- Rapid sequencing from nanogram amounts of DNA template
- Applicablefor experiments requiring mesophilic strand-displacement

Kit Contents

Component	LP301-01-V2 (100 rxns)	LP301-02-V2 (200 rxns)
Bst II DNA Polymerase	200 µl	400 µl
5×LAMP Reaction Mix	0.6 ml	1.2 ml
TS LAMP Green（20×）	100 µl	200 µl
6×DNA Loading Buffer	500 µl	1 ml
Nuclease-free Water	2×1 ml	4×1 ml

Definition of Enzyme Activity

One unit (U) of *Bst* II DNA Polymerase is the amount of enzyme required to catalyze the incorporation of 10 nmol of deoxynucleotides into acid-insoluble substances within 30 minutes at 65°C.

Recommended LAMP Reaction Components (25 µl) based on Fluorescent Dye Method

Component	Volume	Working Concentration
RNA Template	Variable	≥10 copies
FIP/BIP Primers	Variable	1.6 µM
B3/F3 Primers	Variable	0.4 µM
Loop F/B Primers	Variable	0.8 µM
5×LAMP Reaction Mix	5 µl	1×
TS LAMP Green（20×）	0.45 µl	0.36×
Bst II DNA Polymerase	2 µl	640 units/ml
Nuclease-free Water	Variable	-
Total Volume	25 µl	-

Recommended Fluoregenic-Probe-based LAMP Reaction Components (25 μl DARQ-LAMP)

Component	Volume	Working Concentration
DNA Template	Variable	≥10 copies
QPD (Quencher Probe Duplex)-FIP	Variable	The QPD-FIP ratio is 2%,
FIP Primer	Variable	that is, 0.032 μM
BIP Primer	Variable	1.6 μM
B3/F3 Primers	Variable	0.4 μM
Loop F/B Primers	Variable	0.8 μM
5×LAMP Reaction Mix	5 μl	1×
Bst II DNA Polymerase	2 μl	640 units/ml
Nuclease-free Water	Variable	-
Total Volume	25 μl	-

Recommended Reaction Conditions

Incubate at 60℃~65℃ for 30~45 minutes, and set the exposure time to 1 minute. The specific reaction temperature is determined according to the Tm value of the primer. It is recommended to incubate at 85°C for 20 minutes after the reaction to inactivate the *Bst* II enzyme.

Operation Suggestions &Notes

① *Bst* II DNA polymerase does not have 5'→3' exonuclease activity;
② *Bst* II DNA polymerase cannot be used for thermal cycle sequencing or PCR;
③ *Bst* II DNA polymerase reaction temperature range: 50℃~70℃, the optimum reaction temperature is 63℃;
④ The amount of TS LAMP Green dye can be adjusted appropriately, but too high concentration may cause a delay in Ct value;
⑤ Please use Nuclease-free Water or 0.1×TE Buffer to dilute the primers. The concentration ofcomponents in the reaction buffer is high, and the primers diluted with 1×TE may affect the amplification;
⑥ Since *Bst* II DNA polymerase is also active at room temperature, please keep it at a low temperature during the preparation of the reaction mix (operate on ice);
⑦ After preparing the LAMP reaction mix, it is recommended to add a drop of paraffin oil for liquid seal, which can effectively avoid false positives caused by aerosol contamination;
⑧ Try to separate the experimental environment and prepare the reaction reagents and templates in different areas. If you need to perform agarose gel electrophoresis or other analysis methods that require opening the LAMP reaction container after the reaction, please carry out in a separate operating environment to avoid contamination;
⑨ DARQ (Detection of Amplification by Release of Quenching)-LAMP reaction principle and probe design method can refer to Nathan A. Tanner, Yinhua Zhang, and Thomas C. Evans, Jr., Simultaneous Multiple Target Detection in Real-time Loop-mediated Isothermal Amplification. BioTechniques (2012). When using this product for DARQ-LAMP amplification, the optimal addition ratio of the primer QPD-FIP in the probe is 2% of the total amount of the internal primer (1.6 μM), that is, 0.032 μM.

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