Bst III DNA Polymerase

Please read the manual carefully before use.

Cat. No. LP311

Version. No.: Version 1.0

Storage: at -20°C for two years

Description

This product contains *Bst* III DNA polymerase, 5×LAMP Reaction Mix, fluorescent dye TS LAMP Green, and you only need to prepare your own templates and primers. Among them, *Bst* III is an upgraded version of *Bst* II DNA polymerase, which can be used for LAMP reaction with DNA or RNA as template. It is not recommended to add reverse transcriptase when it is used for RT-LAMP reaction.

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 spectrum to 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.

This product is more suitable for RT-LAMP reaction with RNA as template, due to strong reverse transcription activity and amplification ability, which can detect RNA molecules as low as 1 copy within 30 minutes, and can be applied for fluorescent dye/probe method. *Bst* II DNA Polymerase (LP301) is recommended for LAMP amplification of DNA templates.

Highlights

- Isothermal Amplification (LAMP/RT-LAMP) Capability
- Fast polymerization
- Strong strand-displacement capability

Application

- RNA/DNA isothermal amplification
- DNAsequencing through high GC regions
- Applicable for experiments requiring mesophilic strand-displacement

Kit Contents

Component	LP311-01 (100 rxns)	LP311-02 (200 rxns)
Bst III 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

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

Component	Volume	Working Concentration
RNA Template	Variable	≥1 copy
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 III DNA Polymerase	2 µl	-
RNase-free Water	Variable	-
Total Volume	25 µl	-

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

Component	Volume	Working Concentration
RNA Template	Variable	≥1 copy
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 III DNA Polymerase	2 μl	-
RNase-free Water	Variable	-
Total Volume	25 μl	-

Recommended Reaction Conditions

Incubate at 60°C 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 enzyme.

Operation Suggestions &Notes

① Avoid RNase contamination during reaction components preparation;
② *Bst* III DNA Polymerasedoes not have 5'→3' exonuclease activity;
③ *Bst* III DNA polymerase cannot be used for thermal cycle sequencing or PCR;
④ Reaction temperature range: 50 ℃～ 65 ℃, optimum reaction temperature 60 ℃;
⑤ Since *Bst* III 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);
⑥ The amount of TS LAMP Green dye can be adjusted appropriately, but too high concentration may cause a delay in Ct value;
⑦ Please use Nuclease-free Water or 0.1×TE Buffer to dilute the primers. The concentration of components in the reaction buffer is high, and the primers diluted with 1×TE may affect the amplification;
⑧ 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;
⑨ 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;
⑩ 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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