Bst II DNA Polymerase (Lyophilizable Format)

Please read the manual carefully before use.

Cat. No. LP302

Version. No. Version 1.0

Storage: at -20℃ for two years

Description

This product contains high-concentration *Bst* II DNA polymerase and premixed LAMP reaction buffer, suitable for LAMP reaction with DNA as template.

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' exonuclease activity.

5×LAMP Reaction Buffer is an optimized LAMP reaction solution, which already contains $MgSO_4$, dNTPs and other components required for the reaction, and no additional addition is required.

5× LAMP Reaction Buffer does not contain glycero, the glycerol concentration in the stock solution of *Bst* II DNA Polymerase (160 U) is 50%. The reaction system can be used for lyophilization.

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 current procedures and equipment.

Features

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

Application

- DNA isothermal amplification
- DNA sequencing with GC-rich regions
- Applicable for experiments requiring mesophilic strand-displacement

Kit Contents

Component	LP302-01 (100 rxns)	LP302-02 (200 rxns)
Bst II DNA Polymerase(160 U)	10 μl	20 μl
5×LAMP Reaction Buffer	0.5 ml	1 ml
2×Lyophilizing Protectant	1 ml	2×1 ml
TS LAMP Green (20×)	45 μl	90 μl

Lyophilization System

Component	Volume
Bst II DNA Polymerase(160 U)	0.1 μl
5×LAMP Reaction Buffer	5 μl
2×Lyophilizing Protectant	10 μl
RNase-free Water	4.9 μl
Total Volume	20 μl

Recommended Lyophilization Procedure

Step	Stage	Temperature/℃	Slope time /min	Temperature control time /min	Vacuum level /Pa
1	Pre-frozen	-50	0	40	-
2	Sublimation drying	-45	60	120	0
3		-40	30	480	0
4		-30	120	120	0
5	Precipitation drying	26	180	240	0
6	Sample storage	4	0	1440	0

Recommended LAMP System (taking 25 μl fluorescent dye reaction system as an example)

Step	Volume	Final Concentration
Lyophilized Microsphere	Reconstitute with 18 μl RNase-free Water	-
FIP/BIP Primers	Variable	1.6 μM each
F3/B3 Primers	Variable	0.4 μM each
Loop F/B Primers	Variable	0.8 μM each
TS LAMP Green (20×)	0.45 μl	0.36×
DNA Template	Variable	≥10 copies
RNase-free Water	Variable	-
Total Volume		25 μl

Reaction Conditions

Incubate at 60-65°C for 30~45 min, and set the read 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 10-20 min after the reaction to inactivate the enzyme.

Operation Suggestions &Notes

1. Avoid repeated freezing and thawing of all reagent components before lyophilization.
2. When preparing a system for lyophilization, lyophilizing protectant must be added, otherwise it will affect the lyophilization quality.
3. If the excipient formula and liquid components change, it is necessary to re-verify the lyophilizing parameters and make corresponding adjustments to the procedure.
4. Specific requirements for lyophilizing instrument:
   - 4.1 The cold trap temperature (no load) of the freeze dryer ≤ -60°C, the temperature of each plate layer ≤ -50°C, and the temperature uniformity ≤±1°C;
   - 4.2 The instrument has a vacuum adjustment function. Ultimate vacuum degree ≤ 3 Pa, evacuation rate ≤ 30 min (atmospheric pressure to 10 Pa);
   - 4.3 The instrument has a pressure rise test function, and vacuum leakage rate needs to be tested before the lyophilization operation;
   - 4.4 There are differences between different manufacturers and models, and the relevant performance of the machine needs to be fully verified before using.
5. *Bst* II DNA Polymerase lacks 5′→3′ exonuclease activity.
6. *Bst* II DNA polymerase cannot be used for thermal cycle sequencing or PCR.
7. Reaction temperature range: 50 ℃～ 70 ℃; the optimal reaction temperature is 60~65°C.

8. Please use RNase-free Water or 0.1×TE Buffer to dilute the primers. The buffer concentration in the reaction system is high, and the primers diluted with 1×TE Buffer may affect the amplification.
9. 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).
10. After preparing the reaction system, it is recommended to add a drop of paraffin oil for liquid seal, which can effectively avoid false positives caused by aerosol contamination.
11. Try to separate the experimental environment and prepare the reaction reagents and templates in different areas. If agarose gel electrophoresis or other analysis method that require opening the LAMP reaction tube is needed after the reaction, please carry out in a separate operating environment to avoid contamination.

For research use only, not for clinical diagnosis

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