Colorimetric pH Sensitive LAMP Kit (DNA/RNA)

Please read the manual carefully before use

Catalog No. LP321

Storage: at -20°C for one year

Description

This product contains *Bst* II DNA Polymerase, Reverse Transcriptase (High Temperature), 2.5× pH Sensitive LAMP Reaction Mix, and visual pH-sensitive indicator N-Red. Only template and primers need to be prepared by the user. This product is suitable for performing visual LAMP/RT-LAMP reactions using DNA/RNA as templates.

Bst II DNA Polymerase is a recombinant Bacillus stearothermophilus DNA polymerase, purified after expression in Escherichia coli. This enzyme exhibits 5'→3' DNA polymerase activity and lacks 5'→3' exonuclease activity. Reverse Transcriptase (High Temperature) is a high-temperature-tolerant reverse transcriptase, which, when used in combination with *Bst* II, allows for one-step reverse transcription and LAMP amplification using RNA as a template at a constant temperature of 65°C. 2.5× pH Sensitive LAMP Reaction Mix is an optimized pre-mixed reaction solution containing necessary components for LAMP amplification, including $MgSO_4$ and dNTPs. No additional components need to be added during system preparation. N-Red is a pH-sensitive indicator that changes color within the pH range of 6.8 to 8.0. By adding the N-Red color indicator to the LAMP reaction mixture in advance, there is no need to open the lid after the reaction. Visual observation of the color results allows for distinguishing between positive (magenta) and negative (orange-yellow) reaction wells.

Feature

- Isothermal amplification (LAMP/RT-LAMP) capability
- Rapid polymerization
- Strong strand displacement ability

Application

- Isothermal amplification of DNA/RNA
- Nucleic acid sequencing in GC-rich regions
- Suitable for experiments requiring strand displacement at high temperatures

Kit content

Product name	Specification 01 (100 rxns)	Specification 02 (200 rxns)
Bst II DNA Polymerase	100 µl	200 µl
Reverse Transcriptase (High Temperature)	100 µl	200 µl
2.5×pH Sensitive LAMP Reaction Mix	1 ml	2×1 ml
N-red Stain	150 µl	300 µl
RNase-free Water	2×1 ml	4×1 ml

Protocol

1. Target nucleic acid sample preparation

The nucleic acid extracted from samples can be directly added to the reaction as templates.

[Note]: The N-red Stain in this kit is a pH-sensitive indicator. Please avoid using buffer systems with high concentrations of Tris-HCl, as this may prevent the pH indicator from changing color, affecting result interpretation. Avoid using strong acid or strong base nucleic acid releasing agents to process samples. If necessary, after sample processing, adjust the pH to 8.0.

2. Reaction system set up (take 25μl as an example)

Take out each reaction component from -20°C and prepare the reaction system according to the recommended quantities in the table below:

Component	Volume	Working concentration
Template （DNA or RNA）	Variable	＞10 copies
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
2.5×pH Sensitive LAMP Reaction Mix	10 μl	1×
N-Red Stain	1.3~1.5 μl	-
Bst II DNA Polymerase	1 μl	-
Reverse Transcriptase (High Temperature) (add this when template is RNA)	1 μl	-
RNase-free Water	Variable	-
Total Volume	25 μl	

[Note]: ①To avoid contamination, it is recommended to allocate independent areas for component preparation and template addition. Prepare components in a clean bench and add templates in a fume cupboard in another room to prevent false positive results. ②It is suggested to add 20 μl of paraffin oil to each sample well for liquid sealing to prevent reagent evaporation or cross-contamination during the reaction.

3. Isothermal Amplification:

Recommended reaction conditions: React at 60°C to 65°C for 30 minutes. The specific reaction temperature should be determined based on the primer's melting temperature (Tm).

[Note]: It is recommended to incubate at 85°C for 10 minutes after the reaction to deactivate the *Bst* II enzyme.

4. Result Interpretation:

After 30 minutes of reaction, immediately take out the reaction tubes from the reaction machine. After cooling to room temperature, observe the reaction tubes under light (preferably against a white background). A magenta or orange-red color indicates a positive reaction, while yellow indicates a negative reaction. The intensity of color change depends on the amount of template used, with higher template amounts resulting in more noticeable color changes. See data in Figure 1 below.

[Note]: ①After completing the isothermal amplification and 85°C termination steps as described above, samples can be stored at room temperature or 4°C without affecting the color of positive/negative samples. If the 85°C termination step is not performed, the reaction time needs to be accurately timed, and observation and photography should be done immediately. Exceeding the specified time in the instructions may result in false positive results. ②Compared to fluorescent quantitative methods, visual LAMP requires more reaction time to accumulate sufficient products for color change. Therefore, the reaction time depends on the template amount: 30 minutes may be sufficient for high template amounts, while low template amounts may require an extension to 60 minutes.

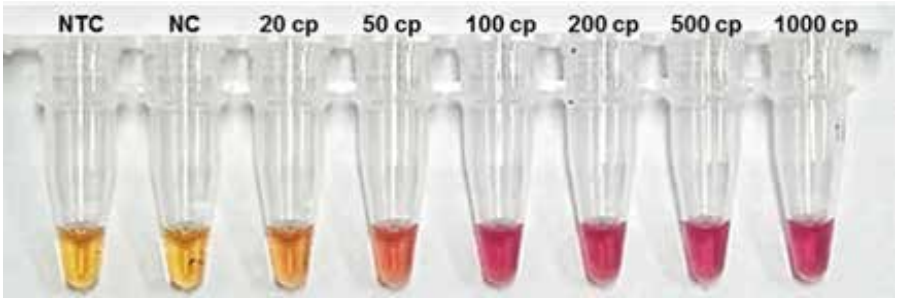

Figure 1. Visualization of LAMP reaction results using N-Red

NTC represents the no-template control, NC represents the negative control, and cp stands for copies, indicating the number of copies per 25 μl reaction system. N-Red volume used: 1.35 μl. Reaction temperature: 65°C. Reaction time: 30 minutes.

Notes

1. *Bst* II DNA Polymerase cannot be used for thermal cycle sequencing or PCR.
2. The reaction temperature range for *Bst* II DNA Polymerase is 50°C to 70°C, with the optimal reaction temperature being 65°C.
3. This product can be used for reactions in water baths, metal baths, PCR machines, or other devices with constant temperature heating modules. It is recommended to add approximately 20 μl of PCR-grade paraffin oil (self-provided) above each sample well after system preparation to effectively prevent reagent evaporation or cross-contamination that may affect result interpretation.
4. The buffering capacity of the buffer solution in this kit is relatively weak. To avoid affecting the color change of the pH indicator, it is recommended to use nucleic acid-free water dissolved templates. When diluting primers, it is recommended to use RNase-free Water or 0.1× TE Buffer.
5. Since *Bst* II DNA Polymerase is active at room temperature, maintain a low temperature (perform on ice) during the system preparation process.
6. Set up independent experimental areas. Prepare reaction reagents and templates in different areas. Do not open the lid after the reaction ends to avoid affecting subsequent experiments.
7. Minimize the number of times the lid is opened. Due to the use of pH indicator method in this product, the buffering capacity of the 2.5× pH Sensitive LAMP Reaction Mix is relatively weak, and each reagent component should not be exposed to the air for a long time. Otherwise, it may adsorb CO2 from the air, resulting in a decrease in pH and affecting experimental results.
8. N-red stain may precipitate during long-term storage, which is normal. Simply mix well before use, and it will not affect the reaction performance.

For research use only, not for clinical diagnosis.

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