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pH-Sensitive Color-Changing Isothermal Amplification Kit (LAMP/RT-LAMP, with UDG)

Product Number: LAP017

Shipping and Storage

Store at -20ºC, valid for one year. LAMP Master Mix with UDG (2×) must be protected from light. Avoid repeated freeze-thaw cycles as much as possible.

Component

Component	100T
LAMP Master Mix with UDG (2×)	1mL
Bst DNA Polymerase	100μL
Positive Control	200μL
Nuclease-free Water	1mL
Mineral Oil	2mL

Description

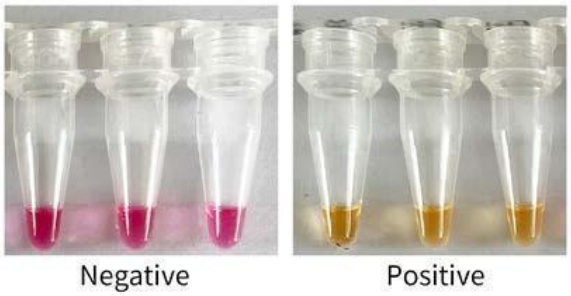
pH-Sensitive Color-Changing Isothermal Amplification Kit (LAMP/RT-LAMP, with UDG) by our company is a kit for amplifying DNA/RNA via Loop-Mediated Isothermal Amplification (LAMP) and detecting the presence of target DNA/RNA in samples through color change of the reaction system. The Bst polymerase in this kit exhibits excellent reverse transcriptase activity, enabling detection of both DNA and RNA templates. This kit incorporates dUTP incorporation and uracil-DNA glycosylase (UDG) technology to prevent contamination from isothermal amplification products, allowing rapid, efficient and highly sensitive detection of target DNA/RNA in samples. It adopts visual colorimetric technology, eliminating the need for electrophoresis, and results can be judged by naked-eye observation of color change. By designing isothermal amplification primers independently, this kit can be used to detect the presence of pathogens, microorganisms, etc. in samples, such as specific pathogen infections or microbial contaminations in biological samples.

Isothermal amplification technology is an in vitro nucleic acid amplification technology that maintains a constant temperature throughout the reaction process and achieves rapid nucleic acid amplification by adding enzymes with different activities and their respective specific primers. This kit employs Loop-Mediated Isothermal Amplification (LAMP), which is characterized by designing 4-6 specific primers targeting different regions of the target gene, using strand-displacement DNA polymerase (Bst DNA Polymerase) to initiate DNA synthesis, forming dumbbell-shaped complementary strands, and further entering the cyclic amplification stage through continuous strand displacement. The final products of amplification are a mixture of DNA with multiple stem-loop structures and cauliflower-like structures. LAMP can complete the nucleic acid amplification reaction only by incubating at an isothermal condition (e.g., 60-65℃) for 30-60 minutes. Compared with conventional PCR, LAMP does not require thermal denaturation of templates, temperature cycling, electrophoresis or ultraviolet observation, and has the advantages of simplicity, rapidity, high sensitivity and strong specificity.

Feature

1. High sensitivity and short reaction time: The sensitivity of this kit is about 10 times higher than that of traditional PCR methods, with a detection limit of approximately 20-200 copies/μL calculated using positive plasmids. The detection reaction can be completed in only 30-60 minutes.
2. Visual colorimetric detection: No electrophoresis is required, and results can be judged by naked-eye observation of color change. This kit contains an optimized red indicator dye. After the target DNA/RNA fragments in the sample are rapidly and massively amplified, the liquid in the reaction tube changes from red or rose red to orange-yellow or yellow, indicating a

positive result for target DNA/RNA. Compared with pH-Sensitive Color-Changing Isothermal Amplification Kit (LAMP/RT-LAMP, with UDG), which changes from violet or blue-violet to sky blue or deep sky blue, this kit shows more



stable color change and more distinct distinction between negative and positive results.

Figure. Detection effect of pH-Sensitive Color-Changing Isothermal Amplification Kit (LAMP/RT-LAMP, with UDG). Left: Negative sample; Right: Positive sample. This figure is for reference only. The colors of negative and positive results may vary slightly between different batches of products. A significant color change between positive and negative samples during actual detection is acceptable.

3. Effective anti-contamination technology: This kit uses dUTP and thermolabile UDG enzyme, which allows dUTP to be incorporated into amplification products. Incubation at 37℃ for 5-15 minutes before isothermal amplification can effectively eliminate product contamination during the isothermal amplification process. During isothermal amplification, the thermolabile UDG enzyme will be inactivated, thus not interfering with subsequent isothermal amplification detection.
4. All-in-one reaction system: This kit contains Bst polymerase, UDG enzyme, PCR Buffer, dNTPs, dUTP and visual indicator. Only appropriate specific primers, test samples and water need to be added to detect target DNA/RNA. The Bst polymerase in this kit has good reverse transcriptase activity and can efficiently synthesize cDNA at 60-65℃, so no separate reverse transcription step is required for RNA samples.
5. Built-in positive control: A Positive Control is provided to verify the normal performance of the kit. The Positive Control contains positive DNA template and corresponding primers targeting GFP DNA. It is recommended to set up a positive control for each detection.
6. Scalable reaction scale: For a standard 20μL reaction system, the small package can perform 25 detections, the medium package 100 detections, and the large package 500 detections.

Protocol

1. Consumables, Instruments and Reagents to Be Prepared by the User

Water bath or conventional thermal cycler (PCR instrument); DNase-free filter tips, 0.2mL PCR tubes.

2. Sample and Primer Preparation

2.1. Prepare samples containing DNA/RNA. For DNA-containing samples such as serum, cell culture supernatants, trace amounts of cells, viruses, bacteria or fungi, direct detection is possible in most cases; purified DNA samples are also acceptable. For RNA-containing samples, direct detection is possible, or RNA can be extracted according to the corresponding requirements and steps in the RNA extraction kit, and the extracted RNA can be directly used for detection. Animal RNA Extraction Kit (Spin Column), Viral RNA Extraction Kit (Spin Column) or Blood RNA Extraction Kit (Spin Column) are recommended.

Note: The sample addition volume is generally 1/10 of the reaction system or less;

If DNA samples cannot be detected immediately, they can be stored at -20ºC or -80ºC for 1-2 months. RNA samples should be stored at -70ºC after purification as much as possible, and repeated freeze-thaw cycles should be avoided;

If direct detection of biological samples containing DNA/RNA is unsatisfactory, try purifying the DNA/RNA samples before detection;

This kit is sensitive to buffer reagents that affect pH. The added samples should avoid using buffer systems such as Tris-HCl. For directly detected samples, it is recommended to use ultrapure water or double-distilled water for storage; for nucleic

acid-purified samples, it is recommended to use ultrapure water or double-distilled water for elution. Since DEPC-treated water may be slightly acidic, adjust the pH to approximately 8.0 with NaOH solution;

Do not treat samples with strong acid or strong alkali reagents. If necessary, purify the samples with a purification kit after treatment, or adjust the pH of the samples to approximately 8.0.

2.2. Prepare specific LAMP Primer Mix (10×): 16 μM FIP/BIP, 2μM F3/B3, 4μM Loop F/B each (optional). Primers can be designed independently according to the target fragment or using specialized LAMP primer design software.

3. Setup of LAMP Reaction System

3.1. Thaw and mix all required solutions, and place them on an ice bath or ice box.

3.2. Set up the LAMP reaction system at room temperature or on an ice bath with reference to the table below (taking a 20μL reaction system per well as an example). The "Template" in the table refers to the sample, Negative Control or Positive Control. The Negative Control can use the solution for dissolving DNA samples or Nuclease-free Water, and the Positive Control can use positive plasmids, etc. If using the Positive Control provided in the kit, since it already contains specific primers, no LAMP Primer Mix (10×) needs to be added, and an additional 2μL of Nuclease-free Water should be added. It is recommended to set up Negative Control and Positive Control for each detection.

Note: It is usually recommended to divide the laboratory into three separate rooms in sequence: sample pretreatment room, LAMP reaction system preparation room, and amplification product processing room to avoid false positives. Special care should be taken when handling high-concentration positive controls; only diluted positive controls are allowed to enter the LAMP reaction system preparation room.

Component	Volume for Sample	Volume for Positive Control
LAMP Master Mix with UDG (2×)	10μL	10μL
Nuclease-free Water	5μL	7μL
LAMP Primer Mix (10×)	2μL	-
Template	2μL	2μL
Bst DNA Polymerase	1μL	1μL
Total Volume	20μL	20μL

3.3. Mix gently by pipetting up and down or slight vortexing, and centrifuge at room temperature for a few seconds to collect the liquid at the bottom of the tube.

3.4. If using a thermal cycler with a heated lid, start the reaction directly; if using a water bath, add 20μL of Mineral Oil to each tube to prevent water evaporation to the tube cap, which may affect the reaction effect.

4. Reaction Conditions

4.1. React at 37℃ in a water bath or thermal cycler for 5-10 minutes, which can effectively remove environmental contamination caused by the isothermal amplification products of this kit in the reaction system. Note: This step is not required for initial detection; it is also not required if it is confirmed that the caps of the isothermal amplification reaction tubes have never been opened. This step only needs to be performed when the detection environment may be contaminated by the isothermal amplification products of this kit.

4.2. If using a water bath for subsequent reaction, preheat the water bath to 61℃ in advance, then place the reaction tubes with floats and incubate in the water bath for 60 minutes. The temperature fluctuation of the water bath should be controlled within 1℃ as much as possible, otherwise it may affect the color change effect.

4.3. If using a thermal cycler for subsequent reaction, set the program as follows: 61℃ for 60 minutes; 4℃ forever; heated lid temperature: 105℃.

Note: The optimal isothermal amplification temperature varies slightly with different primers and needs to be optimized, usually between 60-65℃; The optimal reaction time varies slightly with different template concentrations and needs to be optimized, usually between 30-60 minutes.

5. Result Interpretation

5.1. Take out the reaction tubes, place them at room temperature, and observe the color change against a white background. Negative result: Red or rose red; Positive result: Orange-yellow or yellow; Refer to Figure for specific color development

effects.

Note: If the template concentration in the sample is high, color change will occur within 30-60 minutes; if the template concentration is low, extend the reaction appropriately to a total of 65-70 minutes. If no color change occurs after extended incubation, the result is negative. Excessively prolonged incubation may cause color change in negative samples, resulting in false positives; Do not open the tube caps after the reaction. Isothermal amplification products are extremely abundant, and aerosols can easily contaminate the experimental environment. After result interpretation, seal the tubes and dispose of them according to laboratory waste disposal requirements. If it is necessary to open the caps for subsequent analytical detection, it is recommended to operate in a relatively isolated laboratory.

Note

1. Ensure all reagents are completely thawed before use, mix gently by inverting up and down, and avoid generating bubbles during mixing.
2. Testing has shown that repeated freeze-thawing for 10 times has no significant effect on the performance of this product, but repeated freeze-thawing should still be avoided as it may cause performance degradation.
3. Isothermal amplification is an ultra-sensitive detection method. Please perform the detection in a standard PCR laboratory as much as possible. The area for setting up isothermal amplification reactions should be kept away from all possible contamination by amplification products. Although this kit adopts UDG enzyme anti-contamination technology, do not open the PCR tube caps. Isothermal amplification products should be sealed and disposed of according to the requirements for post-amplification products to avoid contamination of the experimental environment by aerosols containing ultra-high concentration amplification products.
4. This product contains a red indicator. If the color after adding the sample is significantly different from that of the negative and positive controls before the reaction, the sample should be appropriately diluted, processed with a DNA extraction kit, or the pH of the sample should be adjusted to approximately 8.0. Do not use nucleic acid storage solutions containing Tris or other buffer reagents; it is recommended to store nucleic acids in ultrapure water or double-distilled water.
5. This product has no additional buffer reagents and weak buffering capacity. Prolonged exposure to carbon dioxide in the air will acidify the reagent, affect the reagent color and lead to inaccurate result interpretation. Avoid prolonged exposure to air as much as possible.
6. This isothermal amplification system requires high concentrations of divalent ions. Samples must not contain high concentrations of metal ion chelating agents such as EDTA.
7. It is recommended to use filter tips to prepare the isothermal amplification system to minimize false positives caused by contamination.
8. This product is for scientific research use only by professional personnel. It shall not be used for clinical diagnosis or treatment, nor for food or drug purposes. It shall not be stored in ordinary residential premises.
9. For your safety and health, please wear a lab coat and disposable gloves when operating.