Fluorescent and Colorimetric Isothermal Amplification Kit Series

User's Instruction

Description

Fluorescent and Colorimetric Isothermal Amplification Kit Series are specially developed based on isothermal amplification technology, which are specifically for the detection of the virus. The kits have the following characteristics:

- For the 20 μl reaction system, the maximum sample loading can reach 14 μL.
- Containing both visible and fluorescent dyes. The sample can be amplified with either metal bath or water bath, and the results can be judged by either naked eyes or real-time fluorescence detection.
- Added dUTP-UNG can protect against cross contamination.
- The specificity is higher than that of PCR because isothermal amplification uses 4-6 primers instead of 2.
- Lower false negative rate.
- Non-infectious positive control is provided to facilitate analysis of experimental results.
- The kits are available for research use only. Only qualitative tests can be performed, and cannot be used for quantitative tests.
- The kits are enough for 50 reactions of the 20 μL system.

Developed Kits

- Plasmodium malariae Fluorescent and Colorimetric Isothermal Amplification Kit
- Human Papillomavirus (HPV) Fluorescent and Colorimetric Isothermal Amplification Kit
- Hepatitis B Virus (HBV) Fluorescent and Colorimetric Isothermal Amplification Kit
- Mycobacterium tuberculosis (MTB) Fluorescent and Colorimetric Isothermal

Amplification Kit

Kit Contents

Components	Package
5×RT Isothermal Amplification MasterMix	200 μL (Green Cap)
Bst DNA Polymerase（8U/uL）	50 uL (Red Cap)
20× Isothermal Amplification PrimerMix (specifically designed for each virus)	50 μL (White Cap)
Isothermal Amplification Positive Control (1×10E3 Copies/μL) (specifically designed for each virus)	150 μL (Yellow Cap)
Ultrapure Water	1 mL (Bright Yellow Cap)

Protocol

Preparation: if water bath or metal bath is used, the instrument needs to be opened and adjusted to 65°C before the experiment. If using metal bath, water must also be added in the wells to fill the metal pores and the void between the reaction tubes. The metal bath and bath temperature control are far inferior to the PCR instrument, so pre-experimental confirmation with a positive control and a negative control (water) is necessary.

1. **Sample preparation**
   1) The sample can be purified with a self-selection method and the kit is compatible with most viral DNA extraction kits on the market.
   2) If there are N samples, it is necessary to do at least N + 2 sample preparations, including a positive control (a self-prepared template is added to the target molecule being tested, which undergoes the extraction process together with samples) and a negative control (replace the sample with water). Finally, N + 2 samples are processed together for DNA extraction, resulting in N + 2 DNA samples.
2. **Fluorescent and colorimetric isothermal amplification**
   3) When using this kit for the first time, add 50 μl Bst DNA polymerase into 200 μL 5×RT Isothermal Amplification MasterMix at one time. Gently reverse and mix well, then use 5 μL for each 20 μl reaction
   4) Reaction setup: if N + 2 DNA samples are available, it is preferable to set up N + 4 amplifications, adding a negative control and a positive control. Add the following to each of the N + 4 0.2 ml PCR tubes:

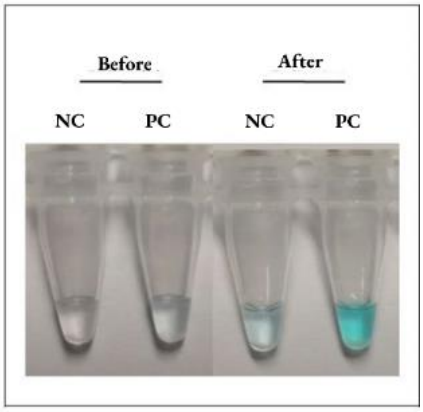
Components	N+2 Samples	Negative Control	Positive Control
5×Isothermal Amplification MasterMix	4 μL	4 μL	4 μL
20× Isothermal Amplification PrimerMix	1 μL	1 μL	1 μL
N+2 Samples	14 μL	-	-
Bst DNA Polymerase	1 μL	1 μL	1 μL
Ultrapure Water		14 μL	-
Isothermal Amplification Positive Control (1×10E3 Copies/μL)	-	-	14 μL

5) Proceed to amplification after mixing. Since this kit contains dual dyes, the following three methods can be chosen for amplification and detection based on laboratory conditions.
6) If the PCR instrument, metal bath, or water bath is used for amplification and detection by the naked eye, 30 μl of self-prepared paraffin oil must first be added to each reaction tube, otherwise the moisture from the reaction system will evaporate during incubation, affecting the efficiency of the reaction (paraffin oil can be omitted if the PCR instrument is used and the hot lid is turned on). 61°C for 90 min, and the liquid color inside the tube can be observed visually.
7) If using a fluorescence quantitative PCR machine: set to 61°C hold for 1 min/cycle, 90 cycles, with fluorescent signal acquired at FAM channel every minute.
8) If the PCR instrument, metal bath or water bath is used for amplification, use the qPCR instrument for endpoint mode fluorescence reading: before and after amplification, measure the fluorescence reading on the qPCR instrument (temperature set to 61°C for 1 cycle of 1 minute each and acquire the fluorescence signal once in the FAM channel. Note: fluorescence readings must be taken at 61°C). Amplification reactions are performed at 65°C for 90 min.

3. Result Analysis

9) If the amplification is performed with a common PCR instrument, water bath or metal bath, the result can only be judged by naked eyes when the reaction is finished. Place the reaction tube on a white background (such as white paper) before observation. Positive controls will appear blue, and negative controls (either no template or no primer) will appear light blue. If the positive control does not appear blue, or if any negative controls appear blue, this illustrates that the reagents are in question and the experiment is not valid. Please contact us in this case.
10) If the experiment is valid, analyze the results from the N + 2 sample tubes.

Amplification is illustrated if the color of the sample tube reaction is close to an amplification positive control tube and no amplification if it is close to a negative control with either no template or no primers. Examples of reaction results are shown below (NC stands for negative control and PC stands for positive control).



11) If amplification is performed with a fluorescent PCR machine, the amplification curve and CT value can be analyzed. The CT value of the positive control provided by the kit should be less than 60. If greater than 60, indicating that the reagent may fail. Please contact us in this case. The CT value of the no template negative control and the no primer negative control should be greater than 90. If any is less than 90, indicate that the reagent or environment is contaminated, please repeat the experiment. If the CT value of both positive and negative controls are within the normal range, the sample can be analyzed by the CT value. The CT value less than 60 is judged positive, greater than 90 is judged negative. If between 60-90, a repeat test is required.
12) If the amplification is performed using a PCR machine, metal bath or water bath, detected visually first, and then using endpoint fluorescence reading to confirm, please compare the positive and negative controls first. The signal increase should be more than 100% after amplification of the positive control sample and less than 50% after amplification of the negative control sample, otherwise the experiment is not valid. Please contact us in this case. If both controls are valid, the difference in fluorescence readings before and after sample amplification can be compared. If the increase in fluorescence signal after amplification is less than 50%, it is judged as negative. If the fluorescence signal increase is higher than 100%, it is judged as positive. If the fluorescence signal increases between 50-100%, it needs to be retested.
13) When both real-time fluorescent detection and visual detection are used for interpretation of results, if they are not in agreement with each other, the data from real-time fluorescent lamp shall prevail. Generally, visualization results lag real-time fluorescence detection results by 20-30 min, that is, samples that are positive at minute 30 on the qPCR instrument and whose color change of reaction fluid is generally not observed until minute 50-60.

Storage

Shipping at low temperature. -20°C for storage with a shelf life of 12 months. Positive controls are recommended to place individually to avoid contaminating other reagents.

Only for research and not intended for treatment of humans or animals