DNA Rapid Isothermal Amplification Kit

(Fluorescent Version)

Product Name

Common Name: DNA Rapid Isothermal Amplification Kit (Fluorescent Version)

Specification

- **Item No.:** WLE8202KIT
- **Specification:** 48 rxns/kit

Description

The DNA Rapid Isothermal Amplification Kit (Fluorescent Type) is developed based on a room-temperature isothermal nucleic acid rapid amplification technology. It is suitable for laboratory-grade DNA amplification and other detection applications requiring DNA amplification. By incorporating template-specific molecular probes, the kit enables real-time monitoring of the target fragment amplification process using fluorescence detection equipment.

Principal

This kit is based on rapid nucleic acid amplification technology that operates at room temperature: under isothermal conditions at room temperature, the recombinase and primers form a Rec/ssDNA complex (protein/single-stranded nucleic acid). With the assistance of accessory proteins and single-stranded binding proteins (SSB), this complex invades the double-stranded DNA template. A D-loop region forms at the invasion site, initiating scanning of the DNA duplex. Once the target region complementary to the primer is identified, the Rec/ssDNA complex disassembles, allowing the polymerase to bind to the 3' end of the primer and initiate extension. This kit operates at 39 ºC and relies on the exonuclease. By incorporating template-specific molecular probes, the kit enables real-time monitoring of the target fragment amplification process using fluorescence detection equipment.

Features and Advantages

- This kit offers high sensitivity, strong specificity, and a rapid reaction time (only takes 20 minutes). The reagents are provided in a lyophilized form for easy handling and storage.
- Operates with basic equipment such as metal bath or water bath, without the need for expensive equipment like PCR machines.

Primer Design

Primer with a length of 30-35 bp is recommended, since too short primer can reduce amplification speed and detection sensitivity. Formation of secondary structures should be avoided during design of primer to ensure the amplification efficient. The length of amplicon is suggested to be 150-300bp, typically not exceeding 500bp.

Fluorescent Probe Design

The probe sequence should have a length of 46-52 nt and should not overlap with the recognition sites of the specific primers. It should avoid palindromic sequences, internal secondary structures, and consecutive repeating bases. The probe includes four modification sites: a dSpacer (tetrahydrofuran, THF) located at least 35 nt from the 5' end, serving as a recognition site for exonuclease; a fluorophore upstream and a quencher downstream of the THF, spaced 2-4 nt apart. The THF site should be at least 15 nt from the 3' end, which is further modified with a group such as an amine, phosphate group, or C3-Spacer.

Storage

- **Shipping Temperature:** Stable temperature of ≤ 20 ºC.
- **Storage Conditions:** Store at ≤ -20 ºC (±5 ºC) in a constant temperature environment, protected from light, and avoid heavy pressure and repeated freeze-thaw cycles.
- **Shelf Life:** 14 months.
- **Production Date:** See the outer packaging.

Kit Component

Component	Volume
A buffer	1.6 mL×1 vial
B buffer	150 μL×1 vial
Positive control template	30 μL×1 vial
Positive control primer-probe mix	70 μL×1 vial
Total	48 rxns
User manual	1 copy

Procedure

Thaw the necessary reagents of the kit at room temperature 30 minutes in advance. Vortex to mix thoroughly.

1. Add 29.4 μL of A buffer to each lyophilized reaction tube.

   Note: Ensure the A buffer thawed and mixed thoroughly, as incomplete mixing may affect the experimental results.

2. Add 2 μL of forward primer, 2 μL of reverse primer and 0.6 μL of probe with a concentration of 10 μM to each reaction tube.

   Note: For multiple reactions, combine steps 1 and 2, then aliquot into the respective tubes.

3. Add 2-13.5 μL of nucleic acid template to the reaction tubes, the volume can be adjusted as needed. Correspondingly adjust the volume of ddH_2O to ensure the total volume of the template and ddH_2O is 13.5 μL.

4. Add 2.5 μL of B buffer to each reaction tube and mix thoroughly.

   Note: Invert the tube 8-10 times to ensure thorough mixing. For multiple reactions, it is recommended to add the B buffer to the inner side of the reaction tube cap, then close the tube and mix by inverting.

5. After mixing, briefly spin down the reaction mixture to the bottom of the tube, or perform a quick centrifugation. Immediately and gently place the reaction tubes in a fluorescence detection device.

Set the detection program as follows: maintain a constant temperature of 39 ºC; collect fluorescence readings from the FAM channel every 30 seconds, ensure that the signal detection channel matches the fluorescent probe design; total reaction time is 20 minutes.

Note: If using an PCR instrument from ABI, be sure to select “none” for both the passive reference and quencher settings.

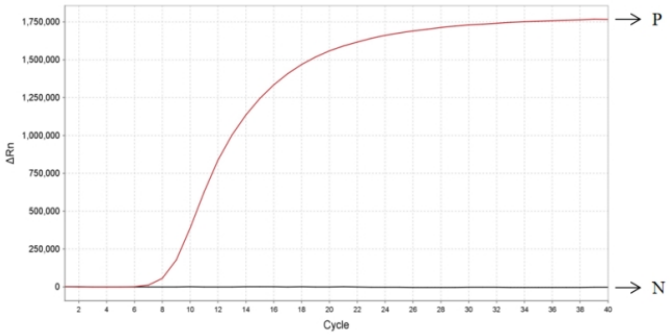
PCR Configuration

Reagent	Volume (μL)
A buffer	29.4
forward primer (10 μM)	2
reverse primer (10 μM)	2
Probe (10 μM)	0.6
ddH_2O and DNA template	13.5
B buffer	2.5
Total Volume	50

Preparation of the Positive Control Reaction System

Add 2 μL of the positive control template and 4.6 μL of the Positive Control Primer-Probe Mix (including the probe and upstream/downstream primers). For other components, please follow the standard protocol.

Positive Control Fluorescence Results Chart



P: Positive Control N: Negative Control

Precautions

- Given the high sensitivity of the kit, it is essential to avoid nucleic acid contamination during the reaction and to include a blank control.

- Only remove the necessary number of MIRA reaction units for your experiment, and store any unused units under the recommended conditions.