DNA Rapid Isothermal Amplification Kit

(Fluorescent Version)

Product Name

DNA Rapid Isothermal Amplification Kit (Fluorescent Version)

Specification

- **Item No.:** EDN-DY01
- **Specification:** 48 rxns

Principal

This kit is based on rapid nucleic acid amplification technology that functions under isothermal conditions at a moderate temperature (typically 39-42 °C). With the assistance of accessory proteins and single-stranded binding proteins, recombinase forms a complex with the primers, which then searches for and binds to the homologous region of the target sequence. A D-loop structure is formed at the homologous site, initiating strand invasion. As the recombinase dissociates from the complex, DNA polymerase binds to the 3' end of the primer and begins strand extension.

Meanwhile, with the involvement of an exonuclease, a sequence-specific molecular probe designed based on the template is introduced. The use of a fluorescence detection system enables real-time monitoring of the amplification process of the target sequence.

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.
- It is compatible with a wide range of fluorescence detection instruments, including real-time

PCR machines and isothermal fluorescence amplification devices from various brands.

Primer Design

It is recommended to use primers with a length of 30-35 bp. Primers that are too short may reduce amplification efficiency and detection sensitivity. Primer design should avoid the formation of secondary structures that could interfere with amplification. The optimal amplicon length is recommended to be between 150 and 300 bp.

Fluorescent Probe Design

The probe sequence should not overlap with the target-binding regions of the specific primers and should be 46–52 nucleotides in length. The sequence should avoid palindromic regions, internal secondary structures, and runs of repetitive bases.

The probe contains four key modifications:

- A dSpacer (tetrahydrofuran, THF) is positioned approximately 30–35 nucleotides from the 5' end and serves as the recognition site for the exonuclease.
- An upstream fluorophore and a downstream quencher are placed on either side of the THF site, with a spacing of 2-4 nucleotides between them.
- The THF site is positioned approximately 15 nucleotides from the 3' end.
- The 3' terminus is blocked with a chemical group such as an amino group, phosphate group, or a C3 spacer to prevent extension.

Storage

- **Shipping Temperature:** Stable temperature of ≤ 20 ℃.
- **Storage Conditions:** Store at ≤ -20 ℃ (±5 ℃) 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	100 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 5 μL of nucleic acid template and 8.5 μL of ddH_2O 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:

   a. B buffer serves as the reaction initiation buffer—once added to the system, the enzymes will be activated.

   b. Be sure to mix by inverting the tube 8-10 times; vortexing or flicking may not achieve

sufficient mixing.

c. For multiple reactions, it is recommended to pre-aliquot the B buffer onto the inner side of each tube cap. After sealing the tubes, invert them to mix. This ensures that all reactions are initiated simultaneously.

5. After mixing, briefly flick the tube or perform a quick centrifugation to bring the reaction mixture to the bottom, then immediately place the tube into the fluorescence detection device.

Set the fluorescence detection program as follows:

- Temperature: Constant at 39-42 °C
- Fluorescence acquisition: Collect FAM channel signal every 30 seconds (the detection channel should match the fluorophore used in the probe design)
- Reaction time: 20 minutes

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

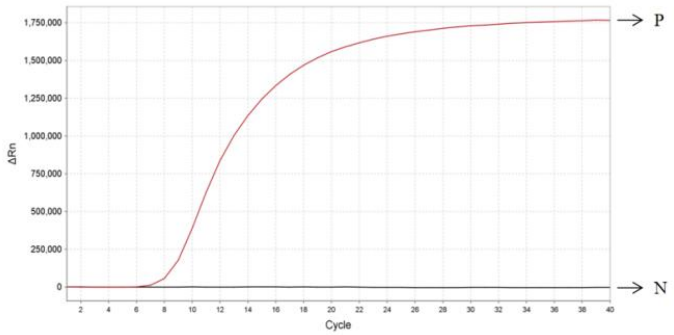
PCR Configuration

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

- Due to the high sensitivity of the kit, please take care to avoid nucleic acid contamination during the reaction, and include a no-template control (NTC) in each run.
- Only take out the number of lyophilized reagents needed for the experiment; store the remaining reagents under the recommended storage conditions.
- Use reagents within their valid shelf life, and do not mix components from different batches.