RNA Rapid Isothermal Amplification Kit (Fluorescent Version) - II

Product Name

RNA Rapid Isothermal Amplification Kit (Fluorescent Version) - II

Specification

- **Item No.:** EDN-RY01
- **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). During the reaction, reverse transcriptase synthesizes a complementary DNA (cDNA) strand using specific DNA primers and an RNA template. With the assistance of accessory proteins and single-stranded binding proteins (SSB), recombinase forms a protein/single-stranded DNA complex (Rec/ssDNA) with the primers, which performs homology searching and binds to the target homologous region. A D-loop structure is then formed at the homologous site, initiating strand exchange. As the recombinase dissociates from the complex, DNA polymerase binds to the 3' end of the primer and begins strand extension.

Simultaneously, relying on the activity of a nuclease, a sequence-specific molecular probe designed based on the template is introduced. Using fluorescence detection equipment, the amplification process of the target fragment can be monitored in real time.

Features and Advantages

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- 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

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 150-500 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 ºC.
- **Storage Conditions:** Store at ≤ -20 ºC (±5 ºC) in a constant temperature environment,

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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
Total	48 rxns
User manual	1 copy

Note: Given the issue of nucleic acid degradation, positive control templates and primers are not provided for RNA series products.

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.

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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 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 RNA template	13.5
B buffer	2.5
Total Volume	50

Precautions

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- 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.

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