RNA Rapid Isothermal Amplification Kit

(Basic Version)

Product Name

RNA Rapid Isothermal Amplification Kit (Basic Version)

Specification

- **Item No.:** EDN-RJ01
- **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 a specific DNA primer and an RNA template. With the assistance of accessory proteins and single-stranded binding proteins (SSB), the recombinase forms a complex with the primer (Rec/ssDNA), which performs homology searching and binds to the target region. A D-loop structure is then formed, initiating strand invasion. As the recombinase dissociates, DNA polymerase binds to the 3' end of the primer and extends the DNA strand. This kit is intended for laboratory-scale RNA amplification and other RNA detection applications in research settings.

Features and Advantages

- This kit offers high sensitivity, strong specificity, and a rapid reaction time (only takes 30 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 150-500bp.

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
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 and 2 μL of reverse primer 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 2-14.1 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 14.1 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 spin down the reaction mixture to the bottom of the tube, or perform a quick centrifugation. Immediately and gently place the reaction tubes in an incubator set at 39-42 ºC for 30 minutes.

6. After the reaction is complete, choose one of the following methods to denature proteins in the reaction product before performing agarose gel electrophoresis (recommended agarose concentration: 1.5-2%):

6.1 Extraction method (recommended):

Add 50 μL of Tris-saturated phenol/chloroform/isoamyl alcohol (25:24:1) DNA extraction reagent to the reaction product. Mix thoroughly, then centrifuge at 12,000 rpm for 5 minutes. Take 5 μL of the supernatant, mix with 1 μL of 6× loading buffer, and load onto an agarose gel for electrophoresis.

6.2 Heat denaturation method:

Add 8 μL of 6× loading buffer directly to the reaction product, mix well, and incubate at 56 °C in a metal bath for 5 minutes. Then load 5 μL of the mixture onto an agarose gel for electrophoresis.

Note:

Using PCR product purification kits to process the reaction product is not recommended. Testing has shown that some commercially available purification kits are unsuitable for this assay and may lead to false-negative results.

PCR Configuration

Reagent	Volume (μL)
A buffer	29.4
forward primer (10 μM)	2
reverse primer (10 μM)	2
ddH_2O and RNA template	14.1
B buffer	2.5
Total Volume	50

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.