RNA Rapid Isothermal Amplification Kit (Basic Version) - II

Product Name

Common Name: RNA Rapid Isothermal Amplification Kit (Basic Version) - II

Specification

- **Item No.:** WLRB8207KIT
- **Specification:** 48 rxns/kit

Description

The RNA Rapid Isothermal Amplification Kit (Basic Version) is developed based on a room-temperature isothermal nucleic acid rapid amplification technology. It is suitable for laboratory-grade RNA amplification and other detection applications requiring RNA amplification.

Principal

This kit is based on a rapid nucleic acid amplification technology that operates under constant temperature of 42 °C. Under these conditions, a specially modified reverse transcriptase utilizes specific primer DNA and template RNA to synthesize cDNA strands. The reaction system includes recombinase, single-stranded DNA-binding proteins, and DNA polymerase, which use the newly synthesized cDNA strands as templates to facilitate rapid nucleic acid amplification reactions.

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.

- 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 150-500bp.

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
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: 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 an incubator set at 42 ºC for 30 minutes.

6. After the reaction, add an equal volume of Tris-saturated phenol/chloroform/isoamyl alcohol in a ratio of 25:24:1 to the reaction mixture. Mix thoroughly in a one-to-one ratio, then centrifuge at 12,000 rpm for 5 minutes. Take 5 μL of the supernatant for agarose gel electrophoresis with a recommended agarose gel concentration of 1.5-2%.

Note:

- High-temperature denaturation may be ineffective at removing proteins, potentially impacting the analysis of results.
- Some commercial kits for purifying amplification products may not be compatible, which could result in false-negative outcomes.

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

- 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.