RNA Rapid Isothermal Amplification Kit (Colloidal gold test strip) - II

Product Name

RNA Rapid Isothermal Amplification Kit (Colloidal gold test strip) - II

Specification

- **Item No.:** EDN-RT01
- **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 from the RNA template using specific primers. With the assistance of accessory proteins and single-stranded binding proteins, the recombinase forms a complex with the primers to search for and bind to homologous regions of the target sequence. 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.

Detection of the amplification product is achieved through a molecular probe specifically designed based on the target sequence. The probe's activity depends on the function of nfo enzyme, and the final result is visualized using colloidal gold technology (sandwich immunoassay format).

Features and Advantages

- This kit offers high sensitivity, strong specificity, and a rapid reaction time (only takes 15 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

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. The 5' end of the downstream primer should be labeled with a modification group, typically biotin. Primer design should avoid the formation of secondary structures that may interfere with amplification. The recommended amplicon length is between 150 and 500 bp.

Fluorescent Probe Design

Design a 46-52 nt sequence complementary to the target region, located between the upstream and downstream primers. The 5' end should be labeled with an antigen tag, typically FAM. A dSpacer (tetrahydrofuran, THF) should be incorporated at an internal position between the 5' and 3' ends, serving as the recognition site for nfo enzyme. The 3' end should be blocked with a modification group, such as an amino group, phosphate group, or C3 spacer.

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, 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 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 8-12 minutes.

6. After the amplification reaction is complete, dilute the product 10-20 times with ddH_2O and

mix thoroughly. Then apply the mixture to the test strip for color development. Observe and interpret the control and test lines within 5 minutes.

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 template	13.5
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.