DNA Rapid Isothermal Amplification Kit

(Colloidal gold test strip)

Product Name

Common Name: DNA Rapid Isothermal Amplification Kit (Colloidal gold test strip)

Specification

- **Item No.:** WLN8203KIT
- **Specification:** 48 rxns/kit

Description

The DNA Rapid Isothermal Amplification Kit (Colloidal gold test strip) is developed based on a room-temperature isothermal nucleic acid rapid amplification technology. It is suitable for laboratory-grade DNA amplification and other detection applications requiring DNA amplification. By incorporating template-specific molecular probes, the kit utilizes colloidal gold technology (sandwich immunoassay) to detect the final results.

Principal

This kit is based on rapid nucleic acid amplification technology that operates at room temperature: under isothermal conditions at room temperature, the recombinase and primers form a Rec/ssDNA complex (protein/single-stranded nucleic acid). With the assistance of accessory proteins and single-stranded binding proteins (SSB), this complex invades the double-stranded DNA template. A D-loop region forms at the invasion site, initiating scanning of the DNA duplex. Once the target region complementary to the primer is identified, the Rec/ssDNA complex disassembles, allowing the polymerase to bind to the 3' end of the primer and initiate extension. Depending on the use of nfo enzyme, incorporating template-specific molecular probes, the kit utilizes colloidal gold technology (sandwich immunoassay) to detect the final results.

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

Primer with a length of 30-35 bp is recommended, since too short primer can reduce amplification speed and detection sensitivity. The 5' end of the downstream primer is labeled with a modification group, typically biotin. Formation of secondary structures should be avoided during design of primer to ensure the amplification efficient. The length of amplicon is suggested to be 150-300bp, typically not exceeding 500bp.

Fluorescent Probe Design

Design a sequence of 46-52 nt complementary to the target fragment between the upstream and downstream primers. Modify the 5' end with an antigen label ad typical FAM. In the central positions of the 5' and 3' ends, include a dSpacer (tetrahydrofuran, THF) as a recognition site for nfo enzyme. At the 3' end, add a modification group, such as an amine, phosphate group, or C3-Spacer.

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

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 2-13.5 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 13.5 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 37-39 ℃ for 8-12 minutes.

6. After completing the reaction, add 10 μL of the reaction mixture to a centrifuge tube containing 190 μL of ddH_2O. Mix thoroughly, then insert the sample end of the colloidal gold test strip into the centrifuge tube and allow it to equilibrate. Observe the control line and the test line to interpret the results 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 DNA 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.