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

Product Name

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

Specification

- **Item No.:** EDN-DT01
- **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). Under the assistance of accessory proteins and single-stranded DNA-binding protein (SSB), recombinase and primers form a Rec/ssDNA complex that performs homology searching and binds to the target homologous region. A D-loop is 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 initiates strand extension. With the action of the endonuclease (nfo), a specific molecular probe designed based on the template is introduced. The final result can be detected using colloidal gold-based sandwich immunoassay technology.

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 300 bp, typically no longer than 500 bp.

Colloidal Gold 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 site near both the 5’ and 3’ ends, serving as the recognition site for nfo enzyme. The 3’ end should be modified with a functional 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
AD buffer	1.6 mL×1 vial
B buffer	150 µL×1 vial
Positive Control Template-IV	100 µL×1 vial
Positive Control Primer-Probe Mix-IV	70 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 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 39-42 ℃ for 10-20 minutes.

6. After the reaction is complete, add 10 μL into a tube containing 190 μL of 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)
AD 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

- Preparation of Positive Control Reaction Mix:

  Add 5 μL of Positive Control Template-IV and 4.6 μL of Positive Control Primer-Probe Mix-IV (already containing the probe and forward/reverse primers). Prepare the remaining components according to the standard reaction system setup.

  Note: The positive control probe in this kit is FAM-labeled, and the reverse primer is Biotin-labeled. Please use a compatible nucleic acid lateral flow strip for detection.

Precautions

- Due to the high sensitivity of this kit, please take care to avoid nucleic acid contamination during operation and always include a no-template control.
- Only remove the required number of lyophilized reagents for the experiment; return the remaining reagents to proper storage conditions promptly.
- Use reagents within their validity period, and do not mix components from different batch numbers.