DNA Isothermal Amplification Kit (Basic Version)

Product Name

DNA Isothermal Amplification Kit (Basic Version)

Specification

- **Item No.:** EDN-DJ01
- **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 binding proteins, recombinase forms a complex with primers, which then performs homology searching and binds to the target homologous region. A D-loop structure is formed at the homologous site, initiating strand invasion. As the recombinase dissociates from the complex, DNA polymerase binds to the 3' end of the primer and begins strand extension.

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

150-300bp, typically not exceeding 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
Positive control template	100 µL×1 vial
Positive control primer mix	60 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 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 5µL 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$_2$O 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 37-39 ℃ 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

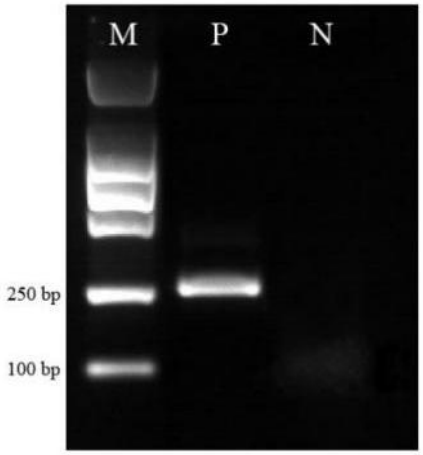
Reagent	Volume (μL)
A buffer	29.4
forward primer (10 μM)*	2
reverse primer (10 μM)*	2

ddH_2O and DNA template	14.1
B buffer	2.5
Total Volume	50

*** Preparation of Positive Control Reaction Mix**

Add 2 μL of the positive control template and 4μL of the positive control primer mix (including both forward and reverse primers). Prepare the remaining components according to the protocol above.

Positive Control Electrophoresis Results



M: Marker P: Positive Control N: Negative Control

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.