Tinzyme Co., Limited

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Long and Accurate PCR Kit

Product Number: PCK56

Shipping and Storage

Storage: -20℃, avoid repeated freezing and thawing.

Components

Component	50T	100T
10×Long PCR Buffer	500μL	1mL
25mM $MgSO_4$ Solution	500μL	1mL
10mM dNTP Mix	60μL	120μL
Dimethyl sulfoxide (DMSO)	250μL	500μL
Long Polymerase(5U/μL)	25μL	50μL
ddH_2O	1mL	2mL

Description

This product is a dedicated kit for one-step reverse transcription real-time fluorescence quantitative detection using the probe method. With extracted RNA as the template, reverse transcription and fluorescence quantitative detection are performed sequentially in the same reaction tube, ensuring simple operation while effectively preventing contamination and reducing pipetting errors. Based on high-efficiency reverse transcriptase, hot-start polymerase, and an optimized buffer system, it delivers excellent amplification performance for RNA templates with complex secondary structures and high GC content, making it highly suitable for detecting trace target genes such as RNA viruses. When using this product, simply add the template, primers, probes, ROX Reference Dye (used to correct fluorescence signal variations between wells, select based on the specific real-time PCR instrument), and water,

Adjust the working concentration to 1× to proceed with the reaction. It offers advantages such as rapid and simple operation, high sensitivity, strong specificity, and excellent stability, effectively minimizing human errors, saving PCR experimental time, and reducing contamination risks.

Application

This kit is used for amplifying fragments greater than or equal to 5kb. The most important component in the reagent kit is Long Polymerase, which is composed of Taq DNA enzyme and thermally stable DNA polymerase with proofreading activity. The main characteristics of this enzyme include two aspects: one is that the product can reach 20kb when using a human genome DNA template, and 40kb when using a viral genome template; the other is higher fidelity than Taq DNA polymerase. Long PCR buffer can protect DNA from damage during long-term thermal cycling.

Protocol

1. **Template DNA:**

   Use 1-10ng of plasmid or phage DNA, or 0.1-1μg of genomic DNA in a 50μL reaction system. High quality and complete template DNA is crucial for long fragment PCR amplification. Damaged DNA may not yield the desired PCR product. Genomic DNA can be obtained through magnetic bead DNA extraction kit without the need for centrifugation process. Additionally, please divide the template DNA into small portions for storage to avoid repeated freezing and thawing. The degraded template DNA is not suitable for expanding the target size.

2. **Primer concentration**

   The final concentration of primers in a PCR is approximately 0.1-0.5μM. When the PCR product is less than or equal to 10kb, the final concentration of the primer is 0.1μM. The design principles for long fragment primers are similar to those for regular primers. The main difference is that the primer should be 28-35 nucleotides in length.

3. Dissolve each component on ice and briefly centrifuge.
4. Prepare the reaction system according to the following experimental plan:

Component	Volume
10×Long PCR Buffer	5μL
10mM dNTP Mix	1μL
Forward primer	0.1～0.5μM
Reverse primer	0.1～0.5μM
Template DNA	1ng～1μg
Long Polymerase	1.25～2.5U
ddH_2O	To 50μL

Note: If the GC content of the PCR product is high, please add 2μL DMSO to 50μL of the PCR reaction system; If the PCR product is greater than 20kb, the final concentration of dNTP Mix is 0.3mM; please add Long Polymerase at the end and start PCR directly. If the PCR product is less than or equal to 15kb, use 1.25U Long Polymerase for 50μL; The PCR product is greater than or equal to 15kb, with a maximum usage of 2.5U per 50μL.

5. Place the reaction tube into a centrifuge and centrifuge for 30-60 seconds;
6. If the thermal cycler used does not have a thermal cap function, cover the PCR mixture with mineral oil.
7. Place the sample into the PCR instrument and start PCR directly according to the following cycle procedure.

Step	Temperature	Time	Cycle
Pre-denaturation	94℃	2min	1
Denaturation	94℃	20sec	10
Annealing	Tm-5℃	30sec	
Extend	68℃	1min/kb	
Denaturation	94℃	20sec	20-25
Annealing	Tm-5℃	30sec	
Extend	68℃	1min/kb+X s/cycle	
Final extension	68℃	10min	1
Save	4℃	N.A.	N.A.

Calculate the extension time according to the following table:

PCR fragment length (kb)	10	15	20	25	30	35	40
Extension time (min)	8	15	20	20	23	25	27
The additional time required for each cycle (X s/cycle)	5	5	10	10	15	15	20

Note

The 10 × Long PCR buffer contains 20mM $MgSO_4$, and the final concentration of $MgSO_4$ in the reaction system is 2mM $MgSO_4$. If a higher concentration of $Mg^{2+}$is required, please adjust the Mg^{2+}concentration using the 25mM $MgSO_4$ solution provided in this kit.

FAQ

1. Few or no products

Insufficient template DNA: Increase the content of template DNA in PCR according to standard operating procedures.

Poor template quality: Always use purified and complete high-quality DNA as a template. The integrity of the template was analyzed by agarose gel electrophoresis. Pack and store template DNA separately to avoid repeated freezing and thawing.

Difficult Template: If the GC content of template DNA is high, adding 4% DMSO to the reaction system will facilitate the reaction. In addition, it is recommended to use enzyme concentrations of up to 2.5U per 50μL PCR reaction system.

Hot cycle related issues: Check if the hot cycle program is set correctly and increase the number of cycles in increments of 5

cycles.

Primer concentration too low: The final concentration of primers used is 0.1-0.5μM.

Mg^{2+}concentration is not optimal: Optimize and adjust the concentration of Mg^{2+}.

2. **Multiple bands of product**

Incomplete primer design: Check primer design and its specificity.

Primer degradation: Check the concentration and quality of primer solution.

Annealing temperature too low: Raise the annealing temperature. Perform gradient PCR to find the optimal annealing temperature.

Too many loops: Reduce the number of cycles to eliminate non-specific products.

Too many templates: When amplifying viral or plasmid DNA, the initial template concentration per 50μL PCR system should not exceed 10ng; When amplifying genomic DNA, the initial template concentration per 50μL PCR system should not exceed 1μg.

3. **The product is contaminated**

Too many starting templates: Reduce the amount of template DNA.

Too many loops: Reduce the number of cycles in increments of 5 cycles

Extended time too long: Reduce extension time in 2-minute increments

Mg^{2+}concentration is not optimal: Optimize and adjust Mg^{2+}concentration

Excessive enzyme: Ensure that the enzyme content added to every 50μL PCR system is equal to or less than 2.5U.

Carryover contamination: Purification of DNA template. Use uracil DNA glycosylation enzyme to prevent contamination.