艾科瑞生物
Accurate Biology

Instruction for Use

ApexHF HS DNA Polymerase CL Master Mix

AG12209
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This Product is optimized for high fidelity PCR (Polymerase Chain Reaction) amplification experiment as a 2X concentration premixed reagent with ApexHF HS DNA Polymerase. This kit is ready for use after simply adding templates and primer sets. This kit is designated for Hot Start PCR with build in Taq Monoclonal Antibody to inhibit Taq DNA Polymerase until reaching the activation temperature. Hot Start PCR greatly reduces non-specific amplification and primer d-dimers.

This kit is suitable for fast and convienient high fidelity PCR experiment and is designated for large and complex DNA fragment PCR. It could be used for high GC ratio, high AT ratio, low template input, extreme large fragment and numerous extreme PCR conditions.

2. Kit Information

Kit Name	Cat. No	Specification
ApexHF HS DNA Polymerase CL Master Mix	AG12209	100 rxns / 50 μl

3. Transportation and Storage

Storage	Store at −20°C
Transportation	Transport at −20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
2X *ApexHF* CL PCR Master Mix	500 μl x 5 pcs

5. Protocol

5.1 Reagent Preparation

The final reaction volume in this protocol is 50μl. The volumes given here may be scaled for larger or smaller reaction volume.

Components	Final Concentration	Volume
2X *ApexHF* CL PCR Master Mix (dye plus)*1	1 X	25 μl
Primer F (10μM)*3	0.2 μM	1 μl
Primer F (10μM)*3	0.2 μM	1 μl
Template	≤ 500 ng*2	-
RNase free water	-	Up to 50 μl

*1: Avoid repeated freeze-thaw cycles of this solution. Before use, centrifuge to collect all liquid at the bottom of the tube to minimize loss. Mix gently (avoid foaming) and pipette slowly during use.

*2:Typically, a template input of less than 500 ng yields optimal amplification results. When using cDNA as the template, it is recommended to use less than 250 ng (equivalent to the total RNA amount for reverse transcription).

*3: Primers are generally used at a final concentration of 0.2 μM. Adjustments can be made within the range of 0.1–0.4 μM based on experimental results.

5.2 Thermal Cycling Program

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results.

Program for 3 Step PCR

Step	Temperature	Time	Number of Cycles
Initial-Denaturation	94°C	1 min*1	1
Denaturation	98°C	10 sec*2	
Annealing	55°C or 60°C*3	5 sec	25~35
Extension	68°C*4	30 sec / kb*5	

*1: For standard templates, the pre-denaturation step can be omitted. For complex templates, such as high-GC or long fragments, pre-denaturation is recommended at 94℃ for 30 sec to 2 min.

*2: Denaturation conditions can be adjusted according to the equipment. Generally, set to 94℃ for 10–15 sec or 98℃ for 5–10 sec.

*3: For annealing, if the Tm value is above 55℃, set the annealing temperature to 60℃; if the Tm value is below 55℃, set it to 55℃. Adjustments can be made based on actual conditions.

*4: The extension speed is generally set to 30 sec/kb. Adjustments can be made between 10 sec and 1 min/kb as needed. For fragments smaller than 10 kb, use 10–30 sec/kb; for fragments larger than 10 kb, use 30 sec to 1 min/kb. For crude extract samples, it is recommended to set the extension speed to 1 min/kb.

*5: For both two-step and three-step PCR methods, the extension temperature can be set to 68℃.

*6: If the primer Tm value is high or three-step PCR results are suboptimal, consider using a two-step PCR method (refer to the appendix for the two-step PCR reaction program).

***5 Program for 2 Step PCR**

Step	Temperature	Time	Number of Cycles
Initial-Denaturation	94°C	1 min	1
Denaturation	98°C	10 sec	
Extension	68°C	30 sec / kb	25~35

6. Result Analysis

Purified PCR product could be analyzed via Agarose Gel Electrophoresis.

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