Instruction for Use

ApexHF HS DNA Polymerase CL

AG12204
Version.V2E1

Research Use Only
Not For Diagnosis Procedures

1. Description

ApexHF HS DNA Polymerase CL is a high-fidelity DNA polymerase with exceptional specificity and amplification performance. It is well-suited for PCR amplification of both simple and complex templates, accommodating short and long DNA fragments. This enzyme is particularly effective for long-fragment PCR, as well as for high-GC, high-AT content templates and low-input DNA samples.

The formulation includes a monoclonal antibody that inhibits enzyme activity at room temperature, enabling Hot Start PCR, which effectively prevents primer dimer formation and non-specific amplification. Additionally, the reaction system has been optimized for robust amplification, even when using crude sample extracts.

2. Kit Information

Kit Name	Cat. No	Specification
ApexHF HS DNA Polymerase CL	AG12204	50 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
ApexHF CL DNA Polymerase CL (1U/μl)	50 μl
5X *ApexHF* CL Buffer (Mg^{2+} plus and dNTP plus)*	1.25 mL

* The Mg^{2+} concentration in 2X ApexHF CL Buffer (Mg^{2} and dNTP plus) is 2 mM, and the dNTP concentration is 400 μM each.

** At 74°C for 30 minutes, using activated salmon sperm DNA as the template/primer, the amount of enzyme required to incorporate 10 nmol of deoxynucleotides into acid-insoluble material is defined as one unit of enzyme activity (U).

5. P.rotocol

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
ApexHF HS DNA Polymerase CL (1U/μl)	1U	1 μl
5X *ApexHF* CL Buffer (Mg^{2+} plus and dNTP plus)	1X	25 μl
Primer F (10μM) *2	0.2 μM	1 μl
Primer R (10μM) *2	0.2 μM	1 μl
Template	≤ 500 ng*3	-
RNase free water	-	Up to 50 μl

*1: Thaw the reagent tube, and vigorously vortex for 30–60 sec to ensure homogeneity before use. Briefly centrifuge to collect contents at the bottom of the tube.

*2: Recommended final concentration for Primer is 0.2μM, could be optimized between 0.1 ~ 0.4μM.

*3: Recommended final concentration is less than 500 ng. When using cDNA as a template, it is recommended to use less than 250 ng, which corresponds to the amount of Total RNA.

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*1	94°C	1 min	1
Denaturation*2	98°C	10 sec	
Annealing*3	55°C or 60°C	15 sec	25~35
Extension	68°C	30 sec / kb*4	

*1: For simple structure template, Initial-Denaturation could be skipped;

For complex template, recommended Initial-Denaturation setup is 94°C for 30 sec-1min.

*2: Could be adjusted per instrument. Recommended to be 94°C for 10-15 sec, or 98°C for 5-10 sec.

*3: If the Tm value is above 55°C, set the annealing temperature to 60°C.

If the Tm value is below 55°C, set the annealing temperature to 55°C.

*4: Recommended extension setup is 30 sec/kb; he extension time can be adjusted within 10 sec to 1 min per kb based on actual conditions.

For fragments smaller than 10 kb, adjust within 10 sec to 30 sec per kb.

For fragments larger than 10 kb, adjust within 30 sec to 1 min per kb.

For crude extraction samples, it is recommended to set the extension speed to 1 min per kb.

*5: 2 Step PCR program (as below) could be adopted for high Tm values or unsatisfying result using 3 Step PCR.

*** Program for 2 Step PCR**

Step	Temperature	Time	Number of Cycles
Initial-Denaturation	94°C	30 sec	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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