



*Instruction for Use*

## ***ApexHFHS* DNA Polymerase FL Master Mix**

AG12207

Version.V3E1

**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 convenient high fidelity PCR experiment and is designated for large DNA fragment PCR, up to 30kb Human gDNA template.

## 2. Kit Information

Kit Name	Cat. No	Specification
<i>ApexHF</i> HS DNA Polymerase FL Master Mix	AG12207	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

**Cat. No AG 12207**

Kit Components	Volume
2X <i>ApexHF</i> FL PCR Master Mix*	500 µl x 5 pcs

\* Mg<sup>2+</sup> concentration is 2 mM, dNTPs concentration is 400 µM.

## 5. Protocol

### 5.1 Reagent Preparation

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

Components	Final Concentration	Volume
2X <i>ApexHF</i> FL PCR Master Mix <sup>*1</sup>	1 X	25 $\mu$ l
Primer F (10 $\mu$ M) <sup>*2</sup>	0.2 $\mu$ M	1 $\mu$ l
Primer R (10 $\mu$ M) <sup>*2</sup>	0.2 $\mu$ M	1 $\mu$ l
Template	$\leq$ 500 ng <sup>*3</sup>	-
RNase free water	-	Up to 50 $\mu$ l

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

\*2: Recommended final concentration for Primer is 0.2  $\mu$ M, could be optimized between 0.1 ~ 0.4  $\mu$ M.

\*3: Recommended final concentration is less than 500 ng. If using cDNA template, recommended final concentration is less than 250 ng.

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

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

For template with High GC ratio or large fragment, recommended 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: When  $T_m$  value > 55°C, annealing temperature shall be 60°C;

For  $T_m$  value < 55°C, annealing temperature shall be 55°C.

\*4: Recommended extension setup is 30 sec/kb, could be adjusted between 5 sec - 1 min/kb.

For fragment  $\leq$  10 kb, the adjustable range is 5 - 30 sec/kb;

For fragment  $\geq$  10 kb, the adjustable range is 30 sec - 1 min/kb.

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

#### \*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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