艾科瑞生物
Accurate Biology

Instruction for Use

ApexHF HS DNA Polymerase FS Master Mix

AG12202
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 convienient PCR experiment with high amplification efficiency, high sensitivity, high specificity, high annealing efficiency and extension speed.

2. Kit Information

Kit Name	Cat. No	Specification
ApexHF HS DNA Polymerase FS Master Mix	AG12202	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 12202

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

* Mg^{2+} concentration is 2 mM, dNTPs concentration is 400 µM.

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* FS PCR Master Mix*1	1 X	25 μl
Primer F (10μM) *2	0.2 μM	1 μl
Primer R (10μM) *2	0.2 μM	1 μl
Template	≤ 200 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 200 ng. For template more than 200 ng, PCR program extension step could be adjusted between 10 - 60 sec /kb.

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	30 sec	1
Denaturation*2	98°C	10 sec	
Annealing*3	55°C	5 or 15 sec	25~35
Extension	72°C	5 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: Annealing Temperature is recommended to be 5 or 15 sec to prevent smear of electrophoresis result.
When Tm value> 55°C, annealing time shall be 5 sec; for Tm value< 55°C, annealing time shall be 15 sec.

*4: Recommended extension setup is 5 sec/kb; extension time could be extended to 10-60 sec/kb for complex templates or unsatisfying trials.

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

***5 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	5 sec / kb	25~35

6. Result Analysis

Purified PCR product could be analyzed via Agarose Gel Electrophoresis.

Accurate Biotechnology (Hunan) Co., Ltd
Hunan Inspection Industrial Park, Bachelor Road,
Yuelu District, Changsha City, Hunan Province, China
service@agbio.com.cn
+86 400 767 6022
en.agbio.com.cn
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