艾科瑞生物
Accurate Biology

Instruction for Use

2X *Accurate Taq* HS PCR Master Mix (UNG plus)

AG11210
Version.V1E1

1. Description

This product incorporates the dUTP/UNG anti-contamination system. In the PCR reaction process, dUTP replaces dTTP. The UNG enzyme selectively hydrolyzes DNA strands containing dU without affecting DNA strands that do not contain dU. This feature removes dU-containing contamination templates introduced during the preparation of the PCR reaction system, effectively preventing false positive PCR results and enhancing result accuracy.

Monoclonal antibodies that inhibit Accurate Taq activity at room temperature are also included in this product, allowing for Hot Start PCR. This effectively suppresses primer dimer formation and non-specific amplification. The PCR products obtained with this product have an A base at the 3' end, facilitating direct cloning into T vectors. Additionally, this product is provided as a 2X PCR reaction premix. Primers, templates, and water need only be added to the premix to perform the PCR reaction. This simplifies the operation, minimizes human error, and allows for rapid detection results within a shorter time frame.

2. Kit Information

Kit Name	Cat. No	Specification
2X *Accurate Taq* HS PCR Master Mix (UNG plus)	AG 11210	200 rxns / 50μl

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 *Accurate Taq* HS PCR Master Mix (UNG plus)	1 ml x 5 pcs
RNase free water	1 ml x 5 pcs

5. Protocol

5.1 Reagent Preparation[*1]

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

Kit Components	Final Concentration	Volume of 50 μl Reaction
2X *Accurate Taq* HS PCR Master Mix (UNG plus)[*2]	1X	25 μl
Primer F (10 μM)	0.2 μM[*3]	1 μl
Primer R (10 μM)	0.2 μM[*3]	1 μl
Template	≤ 500 ng[*4]	-
RNase free water	-	Up to 50 μl

*1: It is recommended that all reaction mixtures be prepared on ice.

*2: Before use, 2X Accurate Taq HS PCR Master Mix (UNG plus) should be briefly centrifuged to collect the solution at the bottom of the tube, minimizing loss.

*3: The final concentration of primers is typically 0.2 μM; adjustments can be made within the range of 0.1 to 1.0 μM as needed.

*4: Generally, the amount of template added should not exceed 500 ng; adjustments can be made according to actual requirements.

5.2 Thermal Cycling Program[*4]

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

Step	Temperature	Time	Number of Cycles
UNG Treatment[*1]	25°C	10 min	1
Initial-Denaturation	95°C	2 min	1
Denaturation[*2]	94°C	30 sec	30
Annealing	55°C	30 sec	
Extension[*3]	72°C	1 min/kb	
Final Extension	72°C	2 min	1

*1. It is recommended to perform UNG treatment at 25°C for 10 minutes to fully degrade contamination templates containing dU. The processing time can be adjusted within the range of 2 to 10 minutes according to actual needs.

*2. PCR denaturation conditions can be set according to the equipment and types of reaction tubes. Generally, it is set at 98°C for 5 to 10 seconds or 94°C for 20 to 30 seconds.

*3. When using dUTP instead of dTTP, PCR amplification efficiency may decrease. If the amplification efficiency is low, it is advisable to extend the extension time appropriately.

*4. In cases where the primer Tm value is high or the results of three-step PCR amplification are unsatisfactory, two-step PCR amplification can be attempted (the procedure for two-step PCR reaction can be found below).

6. Result Analysis

Collect and Purify the PCR product. Then analyse via Agarose Gel Electrophoresis.

Appendix Two Step PCR Program

Step	Temperature	Time	Number of Cycles
UNG Treatment	25°C	10 min	1
Initial-Denaturation	95°C	2 min	1
Denaturation	94°C	30 sec	30
Extension	68°C	1 min/kb	
Final Extension	72°C	2 min	1

Accurate Biotechnology (Hunan) Co., Ltd
No.1 JinYang Road,
WangCheng District, Changsha City, Hunan Province, China
service@agbio.com.cn
+86 400 767 6022
en.agbio.com.cn

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