艾科瑞生物
Accurate Biology

Instruction for Use

SYBR Green Premix Taq HS qPCR Kit (UNG Plus)

AG11756
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a qPCR kit utilizing the SYBR Green I intercalating fluorescence method. It is a 2X premix reagent that simplifies reaction preparation, requiring only the addition of primers, templates, and RNase-free water. Additionally, the kit incorporates the highly efficient Accurate Taq HS DNA polymerase system, enabling high-sensitivity Real-Time PCR reactions for accurate quantification and detection of target genes.

The product also features a dUTP/UNG anti-contamination system. During the PCR reaction, dUTP replaces dTTP, and the UNG enzyme selectively hydrolyzes DNA strands containing dU without affecting DNA strands that lack dU. This effectively eliminates contamination from dU-containing templates introduced during reaction preparation, thereby preventing false-positive PCR results and improving the accuracy of experimental outcomes.

2. Kit Information

Kit Name	Cat. No	Specification
SYBR Green Premix Taq HS qPCR Kit (UNG Plus)	AG 11756	500 rxns / 20 μl

3. Transportation and Storage

Avoid Light Exposure

Storage	Store at –20°C Valid for 6 months while store at 4°C Avoid Direct Light Exposure
Transportation	Transport at –20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
2X SYBR Green Taq HS Premix (UNG Plus)*	1 ml x 5 pcs

*When stored at -20°C, the solution may develop white or light yellow precipitates. Before use, dissolve the solution on ice or by gently warming it in your hand. Mix by inverting the tube until all precipitates are fully dissolved. Avoid vortexing.

5. Protocol

The volumes given here may be scaled for larger or smaller reaction volume. This protocol is given based on the ABI QuantStudio™ 5 Real-Time PCR System. Reaction System and Thermal Cycling Program shall be adjusted per user instrument and experiment.

5.1 Reagent Preparation *1

Components	20 μl Reaction	50 μl Reaction
2X SYBR Green *Pro Taq* HS Premix*2	10 μl	25 μl
Primer F (10μM)*3	0.4 μl	1 μl
Primer R (10μM)*3	0.4 μl	1 μl
ROX Reference Dye (4 μM) *4	0.4 μl	1 μl
Template *5	≤ 100 ng	≤ 250 ng
RNase free water	Up to 20 μl	Up to 50 μl

*1: Prepare the reaction mix according to the recommended system for different instruments.

*2: Avoid repeated freeze-thaw cycles of the product to prevent enzyme activity reduction. Before use, mix gently by inverting the tube; do not vortex. The product contains SYBR Green I, so handle it with care to avoid light exposure.

*3: Primers are typically used at a final concentration of 0.2 μM. If the reaction results are suboptimal, adjust the concentration within the range of 0.1–1.0 μM.

*4: If ROX is required for fluorescence signal calibration, add it according to the amount recommended for the instrument. If ROX is not needed, replace ROX Reference Dye with RNase-free water.

*5: In a 20 μl reaction system, the amount of DNA template added is typically below 100 ng. If necessary, dilute the template DNA to determine the optimal template amount. When using this product for qPCR amplification of cDNA, the volume of cDNA stock solution should not exceed 10% of the total qPCR reaction volume.

5.2 Thermal Cycling Program *1

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

2 Step Thermal Cycling Setup

Step	Temperature	Time	Number of Cycles
UNG Treatment	25°C	10 min*2	1
Pre-Denaturation	95°C	30 sec*3	1
Amplification*5	95°C	5 sec	40
	60°C	30 sec*4	
Melt Curve Collection	Dissociation Stage		

1*: It is recommended to initially adopt the two-step PCR reaction program. If optimal results are not achieved, further optimization of reaction conditions can be performed. If the primer Tm value is low and results in poor amplification efficiency with the two-step method, the three-step method can be used for PCR amplification.

2*: For UNG treatment, it is recommended to process at 25°C for 10 minutes to fully degrade contaminated templates containing dU; The treatment time can be adjusted within a range of 5 to 10 minutes based on actual needs.

3*: The pre-denaturation time is typically set to 30 seconds. If the template is difficult to denature, the pre-denaturation time can be extended to 1–2 minutes.

4*: Under normal circumstances, PCR amplification products are designed to be below 300 bp. When the extension reaction is set to 60°C for 30 seconds, it generally meets the requirements. To improve reaction specificity, the annealing temperature can be increased appropriately. To enhance amplification efficiency or amplify longer PCR products, the extension time can be appropriately extended. The three-step qPCR amplification program may also be tried for such cases (refer to the appendix for the three-step qPCR reaction program).

5*: Fluorescence signal values are collected during this step.

3 Step Thermal Cycling Setup

Step	Temperature	Time	Number of Cycles
UNG Treatment	25°C	10 min	1
Pre-Denaturation	95°C	30 sec	1
Amplification	95°C	5 sec	40
	55°C	30 sec	
	72°C	30 sec*3	
Melt Curve Collection	Dissociation Stage		

6. Result Analysis

Analyse experiment result via amplification curve, melting curve, standard curve per user instrument manual.

Appendix of qPCR Instrument Compatibility Table

Brand	Instrument Model	Rox
Analytik Jena	qTOWER3	-
Agilent	Mx3000P™, Mx3005P™, MX4000™	4 μM
Bioer	Line-Gene	-
Bio-Rad	IQ5, CFX96™, CFX384™, CFX Connect™, MJOpticon, Opticon 2	-
Cepheid	SmartCycler® System, Smart Cycler II System	-
Eppendorf	Mastercycler ep realplex	-
Qiagen	Rotor-Gene® Q, 3000, 6000	-
Roche	LightCycler® 2.0, 480, 96	-
TaKaRa	Thermal Cycler Dice™ TP950	-
Thermo (Life/ABI)	ABI 7500, 7500 Fast, ViiA™7, QuantStudio™ 3/5, QuantStudio™ 6/7/12K Flex, QuantStudio™ Dx	4 μM
Thermo (Life/ABI)	ABI 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOnePlus	20 μM

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