



*Instruction for Use*

## **SYBR Green Premix Pro Taq HS qPCR Kit**

**AG11701**

**Version.V4E1**

**Research Use Only  
Not For Diagnosis Procedures**

### 1. Description

This product is a qPCR reagent kit using SYBR Green I fluorescent dye for detection. It is a 2X premix reagent, making the preparation of the reaction mixture very simple; only primers, templates, and RNase-free water need to be added. The product is optimized for SYBR Green I concentration and PCR reaction system. It uses a Pro Taq HS DNA polymerase system with superior reaction performance, which effectively inhibits the amplification of non-specific products and improves PCR efficiency. It enables high-sensitivity Real-Time PCR reactions, yielding good standard curves within a wide quantitative range for accurate quantification and detection of the target gene.

### 2. Kit Information

Kit Name	Cat. No	Specification
SYBR Green Premix Pro Taq HS qPCR Kit	AG 11701	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 Pro Taq HS Premix*	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

Components	Final Concentration	Volume
2X SYBR Green <i>Pro Taq</i> HS Premix <sup>*1</sup>	1 X	10 µl
Primer F (10µM) <sup>*3</sup>	0.2 µM	0.4 µl
Primer R (10µM) <sup>*3</sup>	0.2 µM	0.4 µl
ROX Reference Dye (4 µM) <sup>*4 *5 *6</sup>	0.08 µM	0.4 µl
Template <sup>*2</sup>	≤ 100 ng	-
RNase free water	-	Up to 20 µl

\*1: Avoid repeated freeze-thaw cycles to prevent enzyme activity loss. Before use, gently invert to mix; do not vortex. The product contains SYBR Green, so take care to avoid light exposure during operation.

\*2: For a 20 µl reaction system, the DNA template amount is typically below 100 ng. If necessary, perform gradient dilution to determine the appropriate template amount. When performing cDNA quantification PCR with this product, the cDNA stock volume should not exceed 10% of the total PCR reaction volume.

\*3: Primers are typically used at a final concentration of 0.2 µM, but can be adjusted within the range of 0.1–1.0 µM.

\*4: Prepare the reaction mixture according to the recommended system for the specific instrument. If ROX is required for fluorescence signal calibration, add the recommended amount as per the instrument guidelines.

\*5: The following products can be used in combination:

ROX Reference Dye (20µM) (AG11703)

ROX Reference Dye (4µM) (AG11710)

Note: The above ROX Reference Dye products are recommended for use at a 50X dilution. If the experimental results are not satisfactory, adjust the amount of ROX Reference Dye added.

\*6: If the PCR instrument does not require ROX, the ROX Reference Dye can be replaced with RNase-free water.

### 5.2 Thermal Cycling Program

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
Pre-Denaturation	95°C	30 sec	1
Amplification	95°C	5 sec	40
	60°C	30 sec	
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 T<sub>m</sub> 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\*: 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.

3\*: 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.

## 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 Cyclus 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


**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

**Research Use Only**