



Instruction for Use

***Pro Taq* HS Premix Probe qPCR Kit**

AG11704

Version.V5E1

**Research Use Only
Not For Diagnosis Procedures**

1. Description

This Product is optimized for probe based Quantitative PCR amplification experiment as a 2X concentration premixed reagent. This kit is ready for use after simply adding templates, primers and probe.

This kit is optimized with *Pro Taq* HS DNA Polymerase system, to efficiently inhibit non-specific amplification and improve amplification result. It is suitable for precise quantification and detection of target genes, acquisition and observation of standard curves in broad molecular biology research aspects.

2. Kit Information

Kit Name	Cat. No	Specification
<i>Pro Taq</i> HS Premix Probe qPCR Kit	AG 11704	500 rxns / 20 μ l

3. Transportation and Storage

Avoid Light Exposure

Storage	Store at -20°C Valid for 6 months while store at 4°C
Transportation	Transport at -20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
2X <i>Pro Taq</i> HS Probe Premix*	1 ml x 5 pcs

*: Might be observed with light yellow or white color precipitation due to -20°C storage. Please dissolve precipitation fully before use. Do not vortex.

5. Protocol

The final reaction volume in this protocol is 20 μ l. The volumes given here may be scaled for larger or smaller reaction volume.

This protocol is given based on the ABI 7500 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 <i>Pro Taq</i> HS Probe Premix* ¹	1 X	10 μ l
Template * ²	≤ 100 ng	-
Primer F (10 μ M)	0.2 μ M * ³	0.4 μ l
Primer R (10 μ M)	0.2 μ M * ³	0.4 μ l
Probe	0.1 ~ 0.5 μ M * ⁴	-
ROX Reference Dye (4 μ M) * ⁵	0.08 μ M	0.4 μ l
RNase free water	-	Up to 20 μ l

*1: Before use, 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. Avoid frequent freeze-thaw. Avoid light exposure.

*2: Recommended final concentration is less than 100 ng. If using cDNA template for quantitative PCR, recommended template volume is no more than 10% (2 μ l) of the system volume (20 μ l).

*3: Recommended final concentration for Primer is 0.2 μ M, could be optimized between 0.1 ~ 1.0 μ M.

*4: Recommended final concentration for Probe is and can be adjust between 0.1 ~ 0.5 μ M. The probe concentration shall be determined per qPCR instruments and fluorescence dye types.

*5: Please check the appendix of qPCR instrument compatibility table for required Rox Reference Dye.

Rox Reference Dye (20 μ M) AG 11703

Rox Reference Dye (4 μ M) AG 11710

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^{*1}

Step	Temperature	Time	Number of Cycles
Step 1	95°C	30 sec ^{*2}	1
Step 2	95°C	5 sec	40
	60°C	30 sec ^{*3}	

*1: Programs could be optimized and altered for unsatisfactory results; If the T_m value of primers is low and causes unsatisfactory result, recommend to re-run with 3 Step Thermal Cycling Program setup.

*2: The pre-denaturation time is recommended to be 30 sec. For complicate structure templates, please extend it to 1 ~ 2 min.

*3: The amplification target shall be designed as <300 bp. To improve amplification specificity, increase the extension temperature; To improve amplification sensitivity and efficiency, prolong the extension running time or re-run with 3 Step Thermal Cycling Program setup.

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 Cyclor 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