艾科瑞生物
Accurate Biology

*Instruction for Use*

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

**AG11733**
**Version.V1E1**

**Research Use Only**
**Not For Diagnosis Procedures**

## 1. Description

This Product is optimized for dye based Quantitative PCR amplification experiment as a 2X concentration premixed reagent. This kit is ready for use after simply adding templates and primer sets. This kit is with tracking functionality for user to visually track plate preparation step to reduce operational errors.

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 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 |
|---|---|---|
| SYBR Green Premix *Pro Taq* HS qPCR Tracking Kit | AG 11733 | 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 |
| Transportation | Transport at –20°C Dry Ice or Blue Ice Condition |

## 4. Kit Components

| Kit Components | Volume |
|---|---|
| 2X SYBR Green *Pro Taq* HS Premix (Blue) | 1 ml x 5 pcs |
| 40 X Dilution Buffer (Yellow) | 500 µl |

## 5. Protocol

The final reaction volume in this protocol is 20μl. 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 *Pro Taq* HS Premix*1 | 1 X | 10 μl |
| Template *2*3 | ≤ 100 ng | - |
| 40 X Dilution Buffer (Yellow)*3*4 | 1 X | 0.5 μl |
| Primer F (10μM)*5 | 0.2 μM | 0.4 μl |
| Primer R (10μM)*5 | 0.2 μM | 0.4 μl |
| ROX Reference Dye (4 μM) *6 | 0.08 μM | 0.4 μl |
| RNase free water | - | Up to 20 μl *7 |

*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 to protect SYBR Green dye.

*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 μl) of the system volume (20 μl).

*3: To conduct tracking function, mix well template and 40 X Dilution Buffer (Yellow) per protocol before being prepared to PCR reaction tubes. If tracking function is not demanded, replace 40 X Dilution Buffer (Yellow) with same volume of RNase free water.

*4: Added volume of 40 X Dilution Buffer (Yellow) shall follow this protocol. Overmuch volume might inhibit amplification efficiency.

*5: Recommended final concentration for Primer is 0.2 μM, could be optimized between 0.1 ~ 1.0 μM.

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

Rox Reference Dye (20μM) AG 11703 Rox Reference Dye (4μM) 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**

| 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 | |
| Step 3 | Dissociation Stage | | |

**3 Step Thermal Cycling Setup**

| Step | Temperature | Time | Number of Cycles |
|---|---|---|---|
| Step 1 | 95°C | 30 sec | 1 |
| Step 2 | 95°C | 5 sec | 40 |
| | 55°C | 30 sec | |
| | 72°C | 30 sec | |
| Step 3 | 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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