



*Instruction for Use*

***Evo M-MLV One Step RT-qPCR Kit II (Probe)***

AG11713

Version.V4E1

**Research Use Only  
Not For Diagnosis Procedures**

## 1. Description

This Product is optimized for One Step Reverse Transcription and Quantitative PCR amplification experiment as the reaction is done within one tube. It is optimized and suitable for experiments with reduced risk of contamination by simplified operational procedures.

This kit is optimized with the *Evo M-MLV* Reverse Transcriptase and *Pro Taq* HS DNA Polymerase system, to efficiently inhibit non-specific amplification, improve amplification result, synthesize cDNA and amplify target DNA high efficiently. It is suitable for precise quantification and detection of target genes, acquisition and observation of standard curves in broad molecular biology research aspects, from RNA templates including micro vial of virus RNA templates.

## 2. Kit Information

Kit Name	Cat. No	Specification
<i>Evo M-MLV</i> One Step RT-qPCR Kit II (Probe)	AG 11713	250 rxns / 20 $\mu$ l

## 3. Transportation and Storage

Storage	Store at $-20^{\circ}\text{C}$
Transportation	Transport at $-20^{\circ}\text{C}$ Dry Ice or Blue Ice Condition

## 4. Kit Components

Kit Components <sup>*1</sup>	Volume
2X One Step RT-qPCR Buffer II (Probe) <sup>*2</sup>	1.25 ml x 2 pcs
<i>Pro Taq</i> HS DNA Polymerase	100 $\mu$ l
<i>Evo M-MLV</i> RTase Enzyme Mix II <sup>*3</sup>	100 $\mu$ l
RNase Free Water	1 ml x 3 pcs

\*1: Probe for qPCR detection is not included and shall be prepared before experiment.

\*2: It contains the dNTP Mixture and Reaction Buffer.

\*3: It contains the *Evo M-MLV* RTase and RNase Inhibitor.

## 5. Precaution and Important Notes

(1) Please adopt adequate operation and lab management methods to control RNase contamination. All lab consumables including centrifuge tubes and pipette tips shall be RNase Free grade.

(2) The enzymes (*Pro Taq* HS DNA Polymerase and *Evo M-MLV* RTase Enzyme Mix II) are highly viscous with high content of glycerin. Please briefly centrifuge and collect the reagent to tube bottom before use.

(3) If precipitation occurs in buffer reagent, please fully dissolve before use.

(4) This kit is designated to be used along with specific RT primers, as not compatible with Random Primer and Oligo dT primer.

## 6. Protocol

The final reaction volume in this protocol is 20 $\mu$ l. 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.

### 6.1 Reagent Preparation <sup>\*1</sup>

Components	Final Concentration	Volume
2X One Step RT-qPCR Buffer II (Probe)	1 X	10 $\mu$ l
<i>Pro Taq</i> HS DNAPolymerase (5U/ $\mu$ l)	2 U	0.4 $\mu$ l
<i>Evo M-MLV</i> RTase Enzyme Mix II	-	0.4 $\mu$ l
Primer F (10 $\mu$ M)	0.2 $\mu$ M <sup>*2</sup>	0.4 $\mu$ l

Primer R (10 $\mu$ M)	0.2 $\mu$ M <sup>*2</sup>	0.4 $\mu$ l
Probe	0.1 ~ 0.8 $\mu$ M <sup>*3</sup>	-
ROX Reference Dye (4 $\mu$ M) <sup>*4</sup>	0.08 $\mu$ M	0.4 $\mu$ l
Template <sup>*5</sup>	$\leq$ 100 ng	-
RNase free water	-	Up to 20 $\mu$ l

\*1: Reaction mix shall be prepared on ice. For multiple reactions, recommend to prepare all reagent master mix firstly, then separately prepare single wells, and lastly add templates.

\*2: Recommended final concentration for Primer is 0.2  $\mu$ M, could be optimized between 0.1 ~ 1.0  $\mu$ M.

\*3: Recommended final concentration for Probe is and can be adjust between 0.1 ~ 0.8  $\mu$ M. The probe concentration shall be determined per qPCR instruments and fluorescence dye types.

\*4: Please check the appendix of qPCR instrument compatibility table for required Rox Reference Dye.

Rox Reference Dye (20 $\mu$ M) AG 11703      Rox Reference Dye (4 $\mu$ M) AG 11710

\*5: Recommended final concentration of template in a 20  $\mu$ l reaction system is less than 100 ng.

## 6.2 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<sup>\*1</sup>

Step	Temperature	Time	Number of Cycles
Reverse Transcription	42°C <sup>*2</sup>	5 min <sup>*2</sup>	1
Pre-Denaturation	95°C	30 sec <sup>*3</sup>	1
Denaturation	95°C	5 sec	40 ~ 45
Annealing and Extension	60°C <sup>*4</sup>	30 sec <sup>*4</sup>	

\*1: Programs could be optimized and altered per the instructions of qPCR instruments.

\*2: The Reverse Transcription reaction is recommended to be 5 min at 42°C. The setup could be optimized and altered per experimental condition.

\*3: The pre-denaturation time is recommended to be 30 sec. For complicate structure templates, please extend it to 1 ~ 2 min.

\*4: The amplification target shall be designed as <300 bp, while the annealing and extension is recommended to be 30 sec at 60°C. 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.

## 7. 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 $\mu$ M
Bioer	Line-Gene	-
Bio-Rad	IQ5, CFX96™, CFX384™, CFX Connect™, MJOpticon, Opticon 2	-
Cepheid	SmartCycler® System, Smart Cyclyer II System	-
Eppendorf	Mastercycler ep realplex	-
Qiagen	Rotor-Gene® Q, 3000, 6000	-
Roche	LightCycler® 2.0, 480, 96	-
TaKaRa	Thermal Cyclyer Dice™ TP950	-
Thermo (Life/ABI)	ABI 7500, 7500 Fast, ViiA™7, QuantStudio™ 3/5, QuantStudio™ 6/7/12K Flex, QuantStudio™ Dx	4 $\mu$ M
Thermo (Life/ABI)	ABI 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOnePlus	20 $\mu$ M



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