艾科瑞生物
Accurate Biology

Instruction for Use

Evo M-MLV One Step RT-qPCR Probe Kit (UNG Plus)

AG11754
Version.V2E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a probe-based, one-step RT-qPCR kit, enabling both reverse transcription and qPCR amplification to be completed in a single tube, offering simple and rapid operation. The kit employs Evo M-MLV Reverse Transcriptase, known for its robust extension capability, and integrates the hot-start Accurate Taq HS DNA Polymerase, enabling efficient cDNA synthesis and stable qPCR amplification within a short time.

This kit offers broad applicability, capable of amplifying targets with varying template complexities, and supports both singleplex and multiplex detection. It is particularly suitable for detecting trace amounts of RNA, such as viral RNA. To ensure high specificity and prevent false positives, this product incorporates a dUTP/UNG anti-contamination system. During PCR, dUTP replaces dTTP, and UNG (uracil-N-glycosylase) selectively hydrolyzes DNA containing dU, eliminating potential contaminants introduced during PCR setup. DNA strands lacking dU remain unaffected. Furthermore, a monoclonal antibody that inhibits DNA polymerase activity at room temperature is included to enable Hot Start PCR, effectively reducing non-specific amplification, enhancing sensitivity, and improving the accuracy of results.

2. Kit Information

Kit Name	Cat. No	Specification
Evo M-MLV One Step RT-qPCR Probe Kit (UNG Plus)	AG 11754	200 rxns / 25 µl

3. Transportation and Storage

Storage	Store at −20°C
Transportation	Transport at −20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
2X One Step RT-qPCR Buffer IV (Probe)	1.25 ml x 2 pcs
One Step Enzyme Mix IV *	400 µl
RNase Free Water	1 ml x 3 pcs

*:This product contains Evo M-MLV RTase Enzyme, Accurate Taq HS DNA Polymerase, UNG Enzyme, 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 glycerinum. 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 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.

6.1 Reagent Preparation *1

Components	Final Concentration	Volume
2X One Step RT-qPCR Buffer IV (Probe)	1 X	12.5 µl
One Step Enzyme Mix IV	-	2 µl

Primer F (10μM)	0.2 μM *2	0.5 μl
Primer R (10μM)	0.2 μM *2	0.5 μl
Probe (10μM)	0.4 μM *3	1 μl
ROX Reference Dye (4 μM) *4	0.08 μM	0.5 μl
Template *5	-	≤ 100 ng
RNase free water	-	Up to 25 μl

*1: Prepare the reaction mixture according to the recommended protocol for your specific instrument.

*2: Primers are typically used at a final concentration of 0.2 μM, but this can be adjusted between 0.1 and 1.0 μM as needed.

*3: Probe concentration depends on the qPCR instrument and type of fluorescent label used; refer to the instrument manual and probe specifications. A final concentration of 0.4 μM is recommended, but it may be adjusted within the 0.1–1.0 μM range.

*4: If ROX is required for fluorescence signal normalization, add it according to the instrument's recommendation; if ROX is not needed, RNase-free water may be used instead of ROX Reference Dye.

*5: In a 25 μl reaction system, RNA template input should typically not exceed 100 ng. Serial dilution is recommended to determine the optimal template input.

*6: This product is highly sensitive; in a 25 μl reaction volume, it is recommended to dilute the template and add 2–5 μl per sample to improve experimental accuracy and reproducibility.

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*1

Step	Temperature	Time	Number of Cycles
UNG Treatment	25°C*2	10 min*2	1
Reverse Transcription	50°C*3	15 min*3	1
Pre-Denaturation	95°C	30 sec*4	1
Denaturation	95°C	5 sec	40 ~ 45
Annealing and Extension*6	60°C*5	30 sec*5	

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

*2: It is recommended to perform UNG treatment at 25°C for 10 minutes to effectively degrade contaminating templates containing dU; the treatment time can be adjusted between 5 to 10 minutes as needed.

*3: The reverse transcription reaction yields optimal results at 50 °C for 15 minutes; however, the reaction time can be adjusted between 5 to 15 minutes based on actual needs to achieve ideal outcomes.

*4: The pre-denaturation step is typically set to 30 seconds, but for difficult-to-denature templates, it can be adjusted between 30 seconds to 5 minutes as needed.

*5: In most cases, PCR amplicons are designed to be under 300 bp, and an annealing/extension condition of 60°C for 30 seconds is sufficient; to enhance specificity, the temperature may be increased, and for higher amplification efficiency or longer amplicons, the annealing/extension time can be extended or a three-step PCR program can be used (refer to the appendix for the three-step protocol).

*6: This step is for fluorescence signal acquisition.

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 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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