

2xGUEasy One Step RT-qPCR Probe U+ Kit

i. Product Description

This kit will efficiently synthesize the first-strand cDNA using genetically modified MMLV Reverse Transcriptase, and the Taq DNA Polymerase which is blocked by antibodies, for quantitative amplification. This kit mainly contains optimized 2xGUEasy RT-qPCR NM Buffer mix, GUEasy RT Enzyme mix (U+, RNasin) and RNase Free ddH₂O, which are added according to the reaction system. The buffer contains Mg²⁺ and dNTP, etc., and the factors that can effectively inhibit non-specific PCR amplification and improve the amplification efficiency of multiple qPCR reaction are added to ensure the amplification efficiency and simultaneously carry out multiple reactions. In addition, UDG / dUTP anti-pollution system ensures the authenticity and reliability of the results even in room temperature. Meanwhile, our deeply optimized multiple reaction buffer system, significantly improves the amplification efficiency, promotes the effective amplification of low concentration template, as well as carries out high-sensitivity reaction.

ii. Advantages

Anti-pollution: UDG / dUTP anti-pollution system ensures the authenticity and reliability of the results.

Fast: one-step method, to detect or quantify the RNA of animals, plants as well as microorganisms quickly and accurately.

Sensitivity: effective for low-copy templates.

Repeatability: Optimized reaction system, ensures repeatability between experiments.

iii. Reaction System

Components	Volume (ul)	Final Conc.
2xGUEasy RT-qPCR NM buffer mix	10	1x
GUEasy RT Enzyme mix (U+, RNasin)	1	-
Probe Mix (10uM)	0.5 each	0.2uM
Primer Mix (10uM)	1 each	0.4uM
Template	1-5ul	-
RNase Free ddH ₂ O	up to 20ul	-

iv. Reference procedure

Cycle steps	Temperature	Time	Number of cycles
Reverse transcription	50°C	5min	1
Initial denaturation	95°C	2-5min	1
Amplification reaction	95°C	5-15s	40
	60°C	30s	

v. Storage condition

Shipped in ice-pack, stored at -20°C, valid for two years.

vi. Product Specification

Product	Catalog #	Size
2xGUEasy One Step RT-qPCR Probe U+ Kit	RTMU3003-1	1 mL
2xGUEasy One Step RT-qPCR Probe U+ Kit	RTMU3003-3	1 mLx3
2xGUEasy One Step RT-qPCR Probe U+ Kit	RTMU3003-5	1 mLx5

vii. Notes

- 1) Be sure to mix well before use, and avoid excessive bubbles caused by violent vibration.
- 2) Primer concentration: Each Primer Mix contains multiple pairs of primers, and usually the final concentration of each primer is 0.25 μ M, it can also be adjusted between 0.1-0.5 μ M according to the application.
- 3) Probe concentration: Each Probe Mix contains multiple probes with different fluorescence signals, and the concentration of each probe can be adjusted between 50-300 nM according to the application;
- 4) Template dilution: Due to the high-sensitive of qPCR, diluting the template is recommended. To control CT value is appropriate between 20 and 35.
- 5) Reaction system: To ensure the effectiveness and repeatability of GOI (gene of interest) amplification, 20 μ L or 50 μ L is recommended.
- 6) Reaction system preparation: To avoid cross-contamination and aerosol-contamination, nuclease-free pipettes (with filter inside) and tubes are recommended, all the reaction system preparation needs to be done in clean bench.
- 7) Annealing/Extension: Reaction temperature and time can be adjusted according to the designed primer T_m value.

viii. Case Study

Chicken liver total RNA quadruple RT-qPCR (FAM █; HEX █; ROX █; Cy5 █)

