2×GUeasy One Step RT-qPCR Probe Kit

i. Product Description

This kit uses genetically modified MMLV Reverse Transcriptase to efficiently synthesize the first-strand cDNA, and uses the Taq DNA Polymerase, which is blocked by antibodies, for quantitative amplification. This kit mainly contains optimized 2×GUeasy RT-qPCR NM buffer mix，GUeasy RT Enzyme mix and RNase Free ddH_2O. It needs to be added according to the reaction system. The buffer contains Mg^{2+} 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.

ii. Advantages

Fast: The one-step method can be used to detect or quantify RNA in animals, plants and 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.
2×GUeasy RT-qPCR NM buffer mix	10	1×
GUeasy RT Enzyme mix	1	-
Probe Mix（10uM）	0.5 each	0.2uM
Primer Mix（10uM）	1 each	0.4uM
Template	1-5ul	-
RNase Free ddH_2O	up to 20ul	-

iv. Reference procedure

Cycle steps	Temperature	Time	Number of cycles
Reverse Transcription	50℃	5min	1
Initial denaturation	95℃	2-5min	1
Amplified reaction	95℃	5-15s	40
	60℃	30s	

v. Storage condition

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

vi. Product Specification

Product	Catalog #	Size
2×GUeasy One Step RT-qPCR Probe Kit	RTM3003-1	1 mL
2×GUeasy One Step RT-qPCR Probe Kit	RTM3003-3	1 mL×3

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 0.25μM, it can also be adjusted between 0.1-0.5 μM 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, 20μL or 50μL 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 Tm value.

viii. Case Study

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