One Step qRT-PCR mastermix 2x UNG+ for probe (FAST)

Enzyme mix (U+)	80 µL
5×RT-qPCR buffer (U+)	0.4 mL

For research use only
Cat No: YT4519
Size: 100 RXN
Store at –20°C

Description

One Step RT-qPCR Probe Kit U+ (Fast) is designed for one-step real-time RT-PCR using probe detection, all RT-PCR steps can be performed in a single tube. This kit can complete 45 cycles of amplification within 40 minutes.
One Step RT-qPCR Probe Kit U+ (Fast) contains heat-labile Uracil N-Glycosylase (UNG), which degrades amplification products from the previous reaction.

Notes:
a. 5×RT-qPCR buffer (U+) includes dNTP and Mg2+; b. Enzyme mix (U+) includes reverse transcriptase, Hot Start Taq DNA polymerase, RNase inhibitor and UDG; c. Use RNase-Free tips, EP tubes, etc.

IMPORTANT
Before use, thoroughly mix the 5×RT-qPCR buffer (U+). If there is any precipitation after thawing, wait for the buffer to return to room temperature, mix and dissolve, and then use them normally.

Reaction setup

Component	**20 µL reactiona**
5×RT-qPCR buffer (U+)	4 µL
Enzyme mix (U+)	0.8 µL
Primer Forward	0.1~1.0 µM
Primer Reverse	0.1~1.0 µM
TaqMan Probe	0.05~0.25 µM
Template	X µL
RNase-Free Water	Up to 20 µL

a. Reaction volume is 10 ~ 50 µL

Cycling protocol: Standard

Step	Cycle	Temprature	Duration Min: sec
Reverse Transcription	1	55℃	10:00
Initial denaturation	1	95℃	00:30
Denaturation	45	95℃	00:10
Annealing&Extension		60℃	00:30

Cycling protocol : Fast

Step	Cycle	Temprature	Duration Min: sec
Reverse Transcription	1	55℃	05:00
Initial denaturation	1	95℃	00:05
Denaturation	43	95℃	00:03
Annealing&Extension		60℃	00:10

a. Reverse transcription temperature is between 50℃ to 60℃, increasing the temperature helps to amplify complex structures and high CG content templates； b. The optimal annealing temperature needs to be adjusted based on the Tm value of the primer, and select the shortest time for fluorescence signal collection based on the Real Time PCR instrument.