MorreRT One Step qRT-PCR Probe Kit

MorreRT One Step qRT-PCR Probe Kit is specially designed for qPCRs that directly use RNA as templates. The reverse transcription and PCR can be finished in one tube, significantly reducing pipetting procedures and the risk of contamination. The MorreRT Reverse Transcriptase and Morretaq hot-start DNA Polymerase contained this kit enables high-sensitive total RNA detection (as little as 1 pg).

The MorreRT One Step qRT-PCR Probe Kit is a master mix system. The 2× One Step Q Probe Mix contains an optimized buffer and dNTP/dUTP mix and is suitable for high-sensitive detection systems based on fluorescence labelled probes (i.e. TaqMan®).

Order Information

Product	Cat. No.	Quantity
MorreRT One Step qRT-PCR Probe Kit	MRTQpb250	250 rxn (20μL/ rxn)

Contents of Kits

Component	Amount
RNase free ddH_2O	1.25 mL x 2
2x One Step Q Probe Mix[a]	1.25 mL x 2
One Step Q Probe Enzyme Mix[b]	250 μL
50x ROX Reference Dye 1[c]	100 μL
50x ROX Reference Dye 2[c]	100 μL

a. Contains dNTP Mix and Mg^{2+}.
b. Contains MorreRT Reverse Transcriptase, RNase inhibitor, and MorreTaq hot-start DNA polymerase.
c. Used to rectify the error of fluorescence signals between different wells.
Use 50× ROX Reference Dye 1 for ABI 7900HT/ 7300 Real-Time PCR and System and StepOne Plus™ ;
Use 50× ROX Reference Dye 2 for ABI 7500, 7500 Fast Real-Time PCR System and Stratagene Mx3000P.
Don't use ROX for neither Roche nor Bio-Rad Real-Time PCR instruments.

Storage

All components should be stored at -20°C.

Additional Materials Required

RNase-free microtube (1.5 mL) or PCR tube (0.2 mL).

PCR instrument or water bath.

Ice bath.

Protocol (Using All StepOne Plus ™)

1. Prepare the reaction solution in a RNase-free PCR tube as follows:

Component	Volume
RNase free ddH_2O	to 20 μL
2x One Step Q Probe Mix	10 μL
One Step Q Probe Enzyme Mix	1μL
50x ROX Reference Dye 1	0.4 μL
Gene Specific Primer Forward (10 μM)[a]	0.4 μL
Gene Specific Primer Reverse (10 μM)	0.4 μL
TaqMan Probe (10 μM)[b]	0.2 μL
RNA Template[c]	Total RNA: 1pg- 1μg

Note: For each component, the volume of can be adjusted according to the following principle:

a. The final concentration of primer is usually 0.2 μM, and if necessary, it can be adjusted between 0.1 μM and 1.0 μM.
b. The final concentration of TaqMan probe can be adjusted between 50 nM and 250 nM.
c. The accuracy of template volumes impacts significant impacts on the qPCR results, due to the high sensitivity this kit. Therefore, to improve experimental repeatability, it is recommended to dilute the template and pipet more volumn to the reaction system.
d. The size of the amplicon should be within the range of 80 bp-200 bp.

2. Place the sample in a qPCR instrument and run the following program for One Step qRT-PCR:

Standard Program

Stage 1	Reverse Transcription	Reps: 1	50°C[a]	15 min
Stage 2	Pre-denaturation	Reps: 1	95°C	30 sec
Stage 3	PCR Cycles	Reps: 45	95°C	10 sec
			60°C	30 sec[b]

Fast Program (suitable for most One Step qRT-PCR)

Stage 1	Reverse Transcription	Reps: 1	50°C[a]	5 min
Stage 2	Pre-denaturation	Reps: 1	95°C	30 sec
Stage 3	PCR Cycles	Reps: 45	95°C	5 sec
			60°C	20 sec[c]

a. For templates with complex secondary structure or high GC-content, the temperature can be increased to 55°C, which will improve the sensitivity and performance.
b. The extension time varies between different qPCR instruments used. For ABI 7700 and 7900HT, the extension time should be ≥ 30 sec; for ABI 7000 and 7300, the extension time should be ≥ 31 sec; for ABI 7500, ≥ 34 sec.
c. Please check the fast program is compatible for the qPCR instrument.

Tips

1. The 2× One Step Q Probe Enzyme Mix contains glycerol. Before pipetting, please collect the liquid by a brief centrifugation.
2. To avoid RNase contamination, please keep the experiment area clean, wear clean gloves and masks, and use RNase-free tubes and tips.