PROBE One-Step RT-qPCR Kit

Código: 13-10507-01 100 reactions

Stored at –20°C.

This kit is stable for 10 days at room temperature.

Description

PROBE One-Step RT-qPCR Kit is designed for quantitative RT-PCR (RT-qPCR) assays in a single tube using fluorogenic probes including TaqMan probes containing M-MLV Reverse Transcriptase RNase H Minus and Hot Start Taq DNA Polymerase.

This kit has been optimized for real-time PCR applications that require high sensitivity and specificity in the detection of RNA targets with fluorogenic probes including the detection and quantification of viral infectious agents and gene expression analysis.

The M-MLV Reverse Transcriptase RNase H Minus is a version of M-MLV RT that has been engineered to reduce RNAse H activity and provide increased thermal stability to synthesize cDNA molecules up to 60°C.

The Hot Start Taq DNA Polymerase is a recombinant Taq DNA polymerase complexed with an anti-Taq monoclonal antibody that blocks the polymerase activity at ambient temperature providing an automatic "hot start" in qPCR for increased sensitivity and specificity detection.

The 2X Reaction Mix is a proprietary buffer containing 6 mM $MgSO_4$, 0.4 mM of each dNTP and stabilizers. This kit also includes a tube of 50 mM $MgSO_4$ for further optimization of the Mg^{2++} concentration in the RT-qPCR.

This kit is compatible with real-time instruments that require low ROX reference dye concentration including Applied Biosystems 7500 / 7500 Fast / ViiA 7 / QuantStudio 12K Flex, Stratagene Mx3000P / Mx3005P / Mx4000 and Agilent AriaMx.

This kit is also compatible with real-time instruments that do not require the ROX reference dye including Roche LightCycler 480, Qiagen Rotor-Gene Q / 3000 / 6000, Eppendorf Mastercycler ep realplex / realplex2 s, Illumina Eco qPCR, Bio-Rad CFX96 / CFX384, BioRad iCycler iQ / MyiQ / iQ5 and Thermo Scientific PikoReal Real-Time PCR.

List of components

Each product contains sufficient reagents to perform 25 µL RT-qPCR reactions.

Código	13-10507-01	100 reactions
50 µL	M-MLV RT / Hot Start Taq Mix	RED CAP
1.25 mL	2X Reaction Mix	BLUE CAP
1 mL	50 mM Magnesium Sulfate (50 mM $MgSO_4$)	WHITE CAP
50 µL	ROX Reference Dye (1.5 µM)	BROWN CAP

Cat. No.	0600.0003.0500	500 reactions
250 µL	M-MLV RT / Hot Start Taq Mix	RED CAP
5 x 1.25 mL	2X Reaction Mix	BLUE CAP
1 mL	50 mM Magnesium Sulfate (50 mM $MgSO_4$)	WHITE CAP
250 µL	ROX Reference Dye (1.5 µM)	BROWN CAP

PROBE One-Step RT-qPCR Kit

Protocol

This protocol is for a reaction size of 25 µL.

The reaction size may be adjusted as desired.

For multiple reaction, prepare a master mix of the components common to all reactions to reduce pipetting errors.

1. Thaw the components at room temperature. When thawed, resuspend the 2X Reaction Mix, 50 mM $MgSO_4$, ROX Reference Dye (optional), primers

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and probe by vortexing and then briefly centrifuge to collect the solution in the bottom of the tube.

In order to maximize specificity, keep all components, reaction mixes and samples on ice. The Hot Start Taq DNA Polymerase is inactive prior to high temperature activation; however, the reverse transcriptase is active at lower temperatures.

2. Prepare the following reaction mix for each sample:

Component	Volume	Final Conc.
2X Reaction Mix	12.5 µL	1X
Forward Primer (40 µM)	0.5 µL	0.8 µM (0.2 – 1 µM)
Reverse Primer (40 µM)	0.5 µL	0.8 µM (0.2 – 1 µM)
Probe (10 µM)	0.5 µL	0.2 µM
ROX Reference Dye (1.5 µM) *optional*	0.5 µL	30 nM
M-MLV RT / Hot Start Taq Mix	0.5 µL	
RNA Template	≤ 10 µL	1 pg to 1 µg total RNA
Nuclease-free water	to 25 µL	

Recommended amplification parameters for RT-qPCR:

2-step cycling protocol

Stage	Step	Temp	Time
Hold	Reverse Transcription	50°C – 60°C	15 – 30 min
Hold	Initial denaturation	95°C	2 min
40 cycles	Denature	95°C	15 sec
	Anneal and Extend	60°C (data collection)	30 – 60 sec

The optimum temperature for reverse transcription step may range from 50°C to 60°C depending on target sequence and reaction conditions.

For most RNA templates, efficient cDNA synthesis can be accomplished in a 15 minutes incubation at 50°C.

For problematic templates containing extensive secondary structure, or to increase the specificity of cDNA priming, increase the cDNA synthesis temperature up to 60°C.

A 3-step cycling protocol (separate annealing and extension steps) may improve assay sensitivity and specificity with some primers/probe sets.

3-step cycling protocol

Stage	Step	Temp	Time
Hold	Reverse Transcription	50°C – 60°C	15 – 30 min
Hold	Initial Denaturation	95°C	2 min
40 cycles	Denature	95°C	15 sec
	Anneal	50°C – 55°C	15 sec
	Extend	72°C (data collection)	45 – 60 sec

Assay Optimization:

PROBE One-Step RT-qPCR Kit performs under a wide range of cycling parameters. The general protocol may be modified to achieve the most sensitive and specific results.

Magnesium concentration:

An optimal magnesium concentration is essentially important to improve the RT-qPCR sensitivity. The 2X Reaction Mix includes magnesium at a final concentration of 3 mM. This works well for most targets; however, the optimal concentration may range from 3 to 6 mM. If necessary, use the separate tube of 50 mM $MgSO_4$ to increase the magnesium concentration. The following table shows the amount of $MgSO_4$ to add to achieve the specified concentration in a 25 µL reaction:

Volume of 50 mM $MgSO_4$	Final $MgSO_4$ Concentration
0.5 µL	4 mM
1 µL	5 mM
1.5 µL	6 mM

Quality Control Assay

This kit is functionally tested in RT-qPCR assays using an Applied Biosystems 7500 Real- Time PCR System following the procedures described in CDC protocol of realtime RTPCR for influenza A (H1N1) 28 April 2009 for the detection of RNaseP gene.

The analysis of real-time RT-qPCR assay should demonstrate the detection of human RNase P gene with a Ct (cycle threshold) < 30 using a synthetic RNA template containing the RNase P gene.

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