Product Manual
Cat. No: #6801

HiPlex qRT-Probe Mix

Description

HiPlex qRT-Probe Mix is an advanced formulated one-step qRT-PCR probe mix for highly sensitive, rapid, and robust detection of RNA target templates. HiPlex qRT-Probe Mix uses state-of-the-art technologies with an antibody-regulated hot-start Taq polymerase and ultra-sensitive reverse transcriptase for efficient cDNA synthesis and real-time PCR amplification in a single reaction chamber or tube. The optimized buffer chemistry and PCR enhancers, RNase inhibitor, and stabilizers enable rapid and sensitive RT-qPCR. HiPlex qRT-Probe Mix is formulated as a 4x mix, which enables extensive multiplexing and means larger volumes of RNA templates can be added to reactions, further enhancing the sensitivity of detection.

HiPlex qRT-Probe Mix is compatible with several probes such as TaqMan® and Scorpions®. This allows rapid detection and quantification of a variety of RNA templates, such as mRNA, viral RNA, and total RNA. The kit includes an efficient thermostable reverse transcriptase with an RNase inhibitor to prevent degradation of RNA templates by RNases.

Kit Components

Component	S pack*	M pack*
HiPlex qRT-Probe Mix (4x)	1 mL	3 x 1 mL
HighScript RTase (20x)	0.2 mL	0.6 mL

*Other pack sizes and bulk orders are available upon request.

Storage and Shipment

Transport with an ice pack. The reagents should be stored at -20°C upon arrival. The reagents are stable until the expiration date if stored correctly. Do not store the mix once it is combined with the RTase.

Reaction Master Mix Set-Up

The recommended master mix set-up for a 20 µL reaction volume is shown in the table below.

Reagent	Volume (µL)	Final concentration
HiPlex qRT-Probe Mix	5	1x
∞Forward primer (10 µM)	X	400 nM-1 µM
∞Reverse primer (10 µM)	X	400 nM-1 µM
∞Probe (10 µM)	X	125-500 nM
20x HighScript RTase	1	1x
RNA template	2-5	Variable
Nuclease-free Water	Up to 20 µL final volume	

∞Primers and probes should be specific to the target DNA/RNA of interest. The recommended Tm for primers is between 56°C and 60°C, and the Tm for probes should be between 65°C and 70°C.

Instrument and Program Set-Up

Cycles	Steps	Temperature	Time
1	[Δ]Reverse Transcription	45–55°C	10 min Singleplex 20 min Multiplex
1	Polymerase activation	95°C	3 min
40	Denaturation	95°C	15 sec
	[ΔΔ]Annealing/ extension	60°C	30 sec

[Δ]The reverse transcription step should be performed at 45°C, except when the RNA template has a complex secondary structure. The reverse transcription time can also be increased to 20 minutes.

[ΔΔ]The annealing/extension step can be reduced to 20 seconds.

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