Ver. 2306-02

RealHelix™ qRT-PCR Kit [Green]

Kit Contents

RealHelix™ qRT-PCR Kit [Green]		
Cat. No.	**QRT2-S200 (200rxns)**	**QRT2-S500 (500rxns)**
QRT Enzyme Mix [Green]	0.4ml	0.5ml x 2ea
QRT 2x Buffer Mix [Green]	1ml x 2ea	1ml x 5ea
ROX Dye (25μM)	0.2ml	0.5ml
Instructions for Use	1ea	1ea

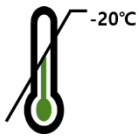
Description

RealHelix™ qRT-PCR kit [Green] is a convenient and reliable one-step quantitative RT-PCR kit. Both cDNA synthesis and PCR reactions are performed in a single tube using sequence-specific primers corresponding to the target RNAs from the sample. The Enzyme Mix in this kit is an optimized blend of Reverse Transcriptase, hot-start PCR enzyme, and RNase inhibitor protein, which ensures reliable results regarding sensitivity, accuracy, and specificity. The 2x Buffer Mix contains all of the required components including optimized buffer components, Mg^{2+}, dNTPs, and a green fluorescent dye. The separately supplied ROX dye could be used as a passive reference dye in real-time PCR instruments that are compatible with the evaluation of the ROX signal.

Application

Quantification of target RNA by real-time PCR

Storage



In the dark at -20℃.

Shelf life

12 months

Quality Control

By Nanohelix's ISO 13485-certified quality management system, each lot of **RealHelix™ qRT-PCR Kit [Green]** was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. **Program a real-time PCR instrument as follows in order to synthesize cDNA and PCR amplification. The excitation and emission maxima of the included green fluorescence dye are at 494 nm and 521 nm, respectively.**

<2-step cycling method>

Step	Condition		Cycle(s)
cDNA Synthesis		50℃ for 10 ~ 40 min	1
PCR Enzyme Activation		95℃ for 15 min	1
PCR Amplification	Denaturation	95℃ for 20 sec	40
	Annealing & Extension	60℃ for 60 sec **Collect the fluorescence data**	

<3-step cycling method>

Step	Condition		Cycle(s)
cDNA Synthesis		50℃ for 10 ~ 40 min	1
PCR Enzyme Activation		95℃ for 15 min	1
PCR Amplification	Denaturation	95℃ for 20 sec	40
	Annealing	[1)]**AT**℃ for 30 sec	
	Extension	72℃ for 1 min/kb **Collect the fluorescence data**	

[1)] **AT,** annealing temperature of primers used

Annealing Temperature = T_m − (6 ~ 8℃)

Where, T_m (Melting Temp.) = [4℃ x (G + C)] + [2℃ x (A + T)]

☞ For melting curve analysis of products, refer the instrument documentation

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2. Add following components for a 20µl volume reaction.

Components	Volumes
RNA Template	X µl
QRT Enzyme Mix [Green]	2.0µl
QRT 2x Buffer Mix [Green]	10µl
Forward primers (10µM)	0.5µl (final 0.25µM)[1)]
Reverse primers (10µM)	0.5µl (final 0.25µM)[1)]
ROX Dye (25µM) [2)]	Optional [3)]
RNase-free Water	Adjust to final 20µl

[1)] The amount of each primer should be adjusted according to the efficiencies of target amplification.

[2)] Avoid freeze/thaw cycles, store dark.

[3)] Use the recommended amount or concentration of ROX Passive Reference Dye depending on the instrument.

3. Gently mix and briefly centrifuge the reaction mix.

4. Perform the Real-time PCR.

Products

Cat. No.	Products	Size
QRT2-S200	RealHelix™ qRT-PCR Kit [Green]	200rxns
QRT2-S500	RealHelix™ qRT-PCR Kit [Green]	500rxns