RealHelix™ qRT-PCR Kit [Probe]

Kit Contents

RealHelix™ qRT-PCR Kit [Probe]		
Cat. No.	**QRT2-P200 (200rxns)**	**QRT2-P500 (500rxns)**
QRT Enzyme Mix [Probe]	0.4ml	0.5ml x 2ea
QRT 2x Buffer Mix [Probe]	1ml x 2ea	1ml x 5ea
ROX Dye (25μM)	0.2ml	0.5ml
Instruction for Use	1ea	1ea

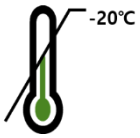
Description

RealHelix™ qRT-PCR Kit [Probe] 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 total RNA or mRNA. The Enzyme Mix in this kit is an optimized blend of HelixCript™ *Thermo* Reverse Transcriptase, RNase inhibitor and a specially designed automatic hot-start version of PCR enzyme, and ensures reliable results in terms of sensitivity, accuracy and specificity. The 2x Buffer Mix contains all of the required components including optimized buffer components, Mg^{2+}, dNTPs. 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

Detection of a target gene transcript from RNA in one-tube format
Absolute and relative quantification analysis of RNA transcription level
Molecular diagnostics for RNA virus

Storage



Store below -20℃
※ ROX Dye should be stored in the dark.

Shelf life

12 months

NanoHelix Co., Ltd.

F711-1(Rev.0)

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Quality Control

By Nanohelix's ISO 13485-certified quality management system, each lot of **RealHelix™ qRT-PCR Kit [Probe]** 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. Set up the excitation and emission maxima suitable to your fluorescent probe chemistry.**

<2-step cycling protocol>

If annealing temperature(AT) of primers used in real-time RT-PCR is between 58℃ and 62℃, thermo cycling can be performed using 2-step cycling protocol for PCR Amplification Step as follow.

Step	Condition		Cycle(s)
cDNA Synthesis		50℃ for 10 ~ 40 min	1
PCR Enzyme Activation		95℃ for 12 ~ 15 min	1
PCR Amplification	Denaturation	95℃ for 20 sec	40
	Annealing & Extension	60℃ for 60 sec **Collect the fluorescence data**	

<3-step cycling protocol>

If annealing temperature(AT) of primers used in real-time RT-PCR is under than 58℃ or above 62℃, thermo cycling can be performed using 3-step cycling protocol for PCR Amplification Step as follow.

Step	Condition		Cycle(s)
cDNA Synthesis		50℃ for 10 ~ 40 min	1
PCR Enzyme Activation		95℃ for 12 ~ 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)]

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☞ Guideline for designing a dual labeled probe

- Check that the probe sequence **contains more C residues** than G residues
- Do not put a **"G"** on 5'-end.
- The optimal probe T_m should be **~ 10℃ higher** than primers

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

Components	Volumes
RNA Template (1ng ~ 1µg)**	X µl
QRT 2x Buffer Mix [Probe]	10µl
QRT Enzyme Mix [Probe]	2µl
Forward primer (10pmoles/µl)	1µl
Reverse primer (10pmoles/µl)	1µl
Fluorescent Probe***	Variable
ROX Dye (25µM)	1 or 0.1µl****
RNase-free Water	Adjust to final 20µl

* For multiple reactions, prepare a master mix by adding the required volumes of each above components (except the RNA template) and dispense appropriate volumes into PCR tubes or plates.

* The reaction volume for a reaction could be adjusted according to the recommendations for the instruments.

** Recommended amount of RNA : Total RNA, 10ng ~ 1µg, Enriched mRNA, 1ng ~ 500ng

*** qRT-PCR Kit [Probe] can be used with various types of fluorescent probes including TaqMan probe and Molecular Beacons probe, etc.

**** Use the recommended amount or concentration of ROX Dye (Passive Reference) depending on the instrument.

3. Gently mix and immediately centrifuge the reaction mix.

4. Perform the Real-time PCR.

Products

Cat. No.	Products	Size
QRT2-P200	RealHelix™ qRT-PCR Kit [Probe]	200rxns
QRT2-P500	RealHelix™ qRT-PCR Kit [Probe]	500rxns

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