

## RealHelix™ qPCR Kit [Probe]

### Kit Contents

RealHelix™ qPCR Kit [Probe]		
Cat. No.	QP2-P200 (200rxns)	QP2-P500 (500rxns)
2x Premix [Probe]	1ml x 2ea	1ml x 5ea
ROX Dye (25µM)	0.2ml	0.5ml
Instruction for Use	1ea	1ea

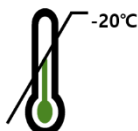
### Description

**RealHelix™ qPCR Kit [Probe]** is designed to perform a rapid real-time quantification of target DNA using dual-labeled probes. The convenient 2x concentrated premix contains hot-start PCR enzyme, dNTPs, buffers, Mg<sup>2+</sup>, and stabilizing agent. The hot-start PCR enzyme provides high specific amplification of target DNA and minimizes the non-specific reactions and production of primer dimers. 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 DNA by real-time PCR

#### Storage



Store below -20°C

※ ROX Dye should be stored in the dark.

#### Shelf life



12 months

## Quality Control

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

## Protocol

1. Program a real-time PCR instrument according to the recommendations below. Set up the excitation and emission maxima suitable to your fluorescent probe chemistry.

### <2-step cycling method>

Step	Condition		Cycle(s)
Enzyme Activation	95°C for 15 min		1
PCR Amplification	Denaturation	95°C for 20 sec	40
	Annealing & Extension	60°C for 40 sec <b>Collect the fluorescence data</b>	

### <3-step cycling method>

Step	Condition		Cycle(s)
Enzyme Activation	95°C for 15 min		1
PCR Amplification	Denaturation	95°C for 20 sec	40
	Annealing	<sup>1)</sup> AT°C for 30 sec	
	Extension	72°C for 1 min/kb <b>Collect the fluorescence data</b>	

<sup>1)</sup> AT, annealing temperature of primers used

$$\text{Annealing Temperature} = T_m - (6 \sim 8^\circ\text{C})$$

$$\text{Where, } T_m \text{ (Melting Temp.)} = [4^\circ\text{C} \times (\text{G} + \text{C})] + [2^\circ\text{C} \times (\text{A} + \text{T})]$$

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

Components	Volumes
DNA Template	X µl
2x Premix [Probe]	10µl
Forward primers (10µM)	0.5µl (final 0.25µM) <sup>1)</sup>
Reverse primers (10µM)	0.5µl (final 0.25µM) <sup>1)</sup>
Probes (10µM)	0.2 ~ 0.5µl (final 0.1 ~ 0.25µM) <sup>1)</sup>
ROX Dye (25µM)	Optional <sup>2)</sup>
RNase-free Water	Adjust to final 20µl

<sup>1)</sup> The amount of each primer and probe should be adjusted according to the efficiencies of target amplification and/or signal intensities.

<sup>2)</sup> Use the recommended amount or concentration of ROX Passive Reference Dye depending on the instrument.

**3. Gently mix and immediately centrifuge the reaction mix.**

**4. Perform the Real-time PCR.**

## Products

Cat. No.	Products	Size
QP2-P200	RealHelix™ qPCR Kit [Probe]	200rxns
QP2-P500	RealHelix™ qPCR Kit [Probe]	500rxns