Ver. 2105-00

RealHelix™ 1-sec qPCR Kit [Probe] (Ver.2.0)

Kit contents

RealHelix™ 1-sec qPCR Kit [Probe] (Ver.2.0)		
Cat. No.	**SQP2-P200** (200 rxns)	**SQP2-P500** (500 rxns)
1-sec 2x Premix [Probe] (V2)	1 ml x 2ea	1 ml x 5ea
ROX Dye (25μM)	0.2 ml	0.5 ml
Instruction for Use	1 ea	1 ea

Description

RealHelix™ 1-sec qPCR Kit [Probe] (Ver.2.0) is designed to perform fast real-time analysis of DNA samples using the fluorescent probe based detection. This kit provides 2x premix and separate ROX reference dye. The convenient 2x concentrated premix contains an antibody-inhibited hot-start *Taq* polymerase (*Ab+Taq* polymerase), dNTPs, buffers, Mg^{2+}, and a stabilizing agent. The premix can also be used in combination with ROX reference dye in PCR instruments that are compatible with the evaluation of the ROX signal. The outstanding fast real-time assay (between 30-60 min.) combined with high specificity and sensitivity is achieved with a unique buffer system and optimized hot-start polymerase.

Application

Fast quantification of target DNA by real-time PCR

Store -20 ℃

ROX Dye should be stored under dark conditions.

Quality control assay data

Functional analysis

RealHelix™ 1-sec qPCR Kit [Probe] (Ver.2.0) was evaluated by real-time PCR using a human genomic DNA, a set of human gene-specific primers, and a dual-labeled probe with ABI 7500 real-time PCR system (Applied Biosystems, USA) and CFX96™ real-time PCR detection system (Bio-Rad, USA).

Quality authorized by Yountaek Go

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Protocol

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

<2-step cycling protocol>

Step	Condition		Cycle(s)
Enzyme activation		95℃ for 2 ~ 5 min	1
PCR Amplification	Denaturation	95℃ for 1 ~ 10 sec*	40
	Annealing & extension	**55 ~ 60**℃ for 1 ~ 30 sec* **Collect the fluorescence data**	

*** The reaction time for each step should be optimized on the applied thermocycler.**

<3-step cycling protocol>

Step	Condition		Cycle(s)
Enzyme activation		95℃ for 2 ~ 5 min	1
PCR Amplification	Denaturation	95℃ for 1 ~ 10 sec*	40
	Annealing	[1)]**AT** ℃ for 1 ~ 20 sec*	
	Extension	72℃ for 1 ~ 30 sec* **Collect the fluorescence data**	

*** The reaction time for each step should be optimized on the applied thermocycler.**

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

Annealing Temperature = T_m − (4 ~ 6℃)

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

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

Components	Volumes
DNA Template	X µl
1-sec 2x Premix [Probe] (V2)	10 µl
Forward primer (10 µM)	0.5 ~ 1.0 µl
Reverse primer (10 µM)	0.5 ~ 1.0 µl
Fluorescent Probe (5 µM)	0.5 ~ 1.0 µl
ROX Dye (25 µM)	Optional **
DEPC-treated water	Adjust to final 20 µl

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

** 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
SQP2-P200	RealHelix™ 1-sec qPCR Kit [Probe] (Ver.2.0)	200 rxns
SQP2-P500	RealHelix™ 1-sec qPCR Kit [Probe] (Ver.2.0)	500 rxns