RealHelix™ 1-sec qPCR Kit [Probe] [UDG System] (V2B)

Kit Contents

RealHelix™ 1-sec qPCR Kit [Probe] [UDG System] (V2B)		
Cat. No.	**SQPU2B-P200 (200rxns)**	**SQPU2B-P500 (500rxns)**
1-sec 2x Premix [Probe] [UDG System] (V2B)	1ml x 2ea	1ml x 5ea
ROX Dye (25μM)	0.2ml	0.5ml
Instructions for Use	1ea	1ea

Description

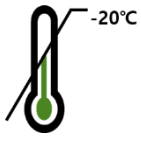
RealHelix™ 1-sec qPCR Kit [Probe] [UDG System] (V2B) is designed to perform a rapid real-time quantification of DNA samples using the fluorescent probe based detection. The UDG/dUTP system prevents the carryover contamination of PCR products from previous reactions.

The 2x premix contains antibody-inhibited hot-start *Taq* polymerase, thermo-labile Uracil-DNA glycosylase, dUTP, dNTPs, buffers, Mg^{2+} and a stabilizing agent. The hot-start PCR enzyme provides high specific amplification of target DNA and minimizes the side products such as primer dimers.

Application

Quantification of target DNA sample by real time PCR

Storage



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

Shelf life

12 months

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Quality Control

By Nanohelix's ISO 13485-certified quality management system, each lot of **RealHelix™ 1-sec qPCR Kit [Probe] [UDG System] (V2B)** was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. **Program a real-time PCR instrument according to the recommendations below.**

<2-step cycling protocol>

If annealing temperature (AT) of primers used in real-time PCR is between 55℃ and 60℃, thermo cycling can be performed using 2-step cycling protocol as follow.

Step	Condition		Cycle(s)
[Optional] UDG reaction*		20 ~ 25℃ for 5 min	1
Enzyme Activation		95℃ for 2 ~ 5 min	1
PCR Amplification	Denaturation	95℃ for 1 ~ 10 sec**	45
	Annealing & Extension	**55 ~ 60**℃ for 1 ~ 30 sec** **Collect the fluorescence data**	

* The UDG reaction step is not essential. The UDG will efficiently remove carryover contaminant DNA during sample setup and cycler ramping.

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

<3-step cycling protocol>

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

Step	Condition		Cycle(s)
[Optional] UDG reaction*		20 ~ 25℃ for 5 min	1
Enzyme Activation		95℃ for 2 ~ 5 min	1
PCR Amplification	Denaturation	95℃ for 1 ~ 10 sec**	45
	Annealing	**AT[1)]**℃ for 1 ~ 20 sec**	
	Extension	72℃ for 1 ~ 30 sec** **Collect the fluorescence data**	

* The UDG reaction step is not essential. The UDG will efficiently remove carryover contaminant DNA during sample setup and cycler ramping.

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

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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)]

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

Components	Volumes
DNA Template	X µl
1-sec 2x Premix [Probe] [UDG System] (V2B)	10.0µl
Forward primer (10µM)	0.5 ~ 1.0µl
Reverse primer (10µM)	0.5 ~ 1.0µl
Fluorescent Probe (5µM)	0.5 ~ 1.0µl
ROX Dye (25µM)	Optional **
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 DNA template) and dispense appropriate volumes into PCR tubes or plates.

** Use the recommended amount 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
SQPU2B-P200	RealHelix™ 1-sec qPCR Kit [Probe] [UDG System] (V2B)	200rxns
SQPU2B-P500	RealHelix™ 1-sec qPCR Kit [Probe] [UDG System] (V2B)	500rxns

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