

## RealHelix™ 1-sec qRT-PCR Kit [Probe] [UDG System] (Ver. 2B)

### Kit Contents

#### RealHelix™ 1-sec qRT-PCR Kit [Probe] [UDG System] (Ver. 2B)

Cat. No.	SQRU2B-P200 (200rxns)	SQRU2B-P500 (500rxns)
1-sec qRT-PCR 2x Premix [Probe] [UDG] (V2B)	1ml x 2ea	1ml x 5ea
ROX Dye (25µM)	0.2ml	0.5ml
Instructions for Use	1ea	1ea

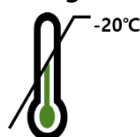
### Description

**RealHelix™ 1-sec qRT-PCR Kit [Probe] [UDG System] (Ver. 2B)** is a 2x premix for qRT-PCR assay using fluorescent probe-based detection. This premix effectively delivers reproducible, reliable detection of up to five RNA targets by fast multiplex in a single tube reaction (FAST: 45 min/40 cycles or ULTRAFast: 25 min/40 cycles). The combination of enzymes (antibody-inhibited hot-start *Taq*, reverse transcriptase, RNase inhibitor, heat-labile UDG) and Nanohelix's unique buffers (including dNTP(U)s, Mg<sup>2+</sup>, and a stabilizing agent) in the ready-to-use premix provides outstanding speed, specificity, and sensitivity of the real-time assay. The applied UDG system prevents the carryover contamination of products from previous reactions.

### Application

Quantification of target RNA By real-time RT-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™ 1-sec qRT-PCR Kit [Probe] [UDG System] (Ver. 2B)**, was tested against predetermined specifications to ensure consistent product quality.

## Protocol

1. Program a real-time PCR instrument as follows to synthesize cDNA and PCR amplification. Set up the excitation and emission maxima suitable to the fluorescent probe chemistry.

### Standard reaction\*

Step	Condition	Cycle(s)
[Optional] UDG Reaction**	20 ~ 25°C for 5 min	1
cDNA Synthesis	50°C for 30 min	1
PCR Enzyme activation	95°C for 5 min	1
PCR Amplification	Denaturation	95°C for 15 sec
	Annealing & Extension	60°C for 1 min <b>Collect the fluorescence data</b>

### Fast reaction\*

Step	Condition	Cycle(s)
[Optional] UDG Reaction**	20 ~ 25°C for 5 min	1
cDNA Synthesis	50°C for 10 min	1
PCR Enzyme activation	95°C for 2 ~ 5 min	1
PCR Amplification	Denaturation	95°C for 1 ~ 10 sec
	Annealing & Extension	60°C for 1 ~ 30 sec <b>Collect the fluorescence data</b>

\* The reaction conditions could be modified and optimized to the system used.

\*\* The UDG reaction step is not essential because UDG can efficiently remove the carryover contaminant DNA during the preparation of the RT-PCR mixture and cyler ramping.

**2. Add the following components in a PCR tube for a single 20 $\mu$ l reaction.**

Components	Volumes
Template RNA	5.0 $\mu$ l
1-sec qRT-PCR 2x Premix [Probe] [UDG] (V2B)	10.0 $\mu$ l
Forward primer (5 $\mu$ M)	1.0 $\mu$ l
Reverse primer (5 $\mu$ M)	1.0 $\mu$ l
Probe (2.5 $\mu$ M)	1.0 $\mu$ l
ROX Dye (25 $\mu$ M)	Optional*
RNase-free Water	Adjust to final 20 $\mu$ l

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

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

**4. Perform real-time RT-PCR.**

## Products

Cat. No.	Products	Size
<b>SQRU2B-P200</b>	RealHelix™ 1-sec qRT-PCR Kit [Probe] [UDG System] (Ver. 2B)	200rxns
<b>SQRU2B-P500</b>	RealHelix™ 1-sec qRT-PCR Kit [Probe] [UDG System] (Ver. 2B)	500rxns