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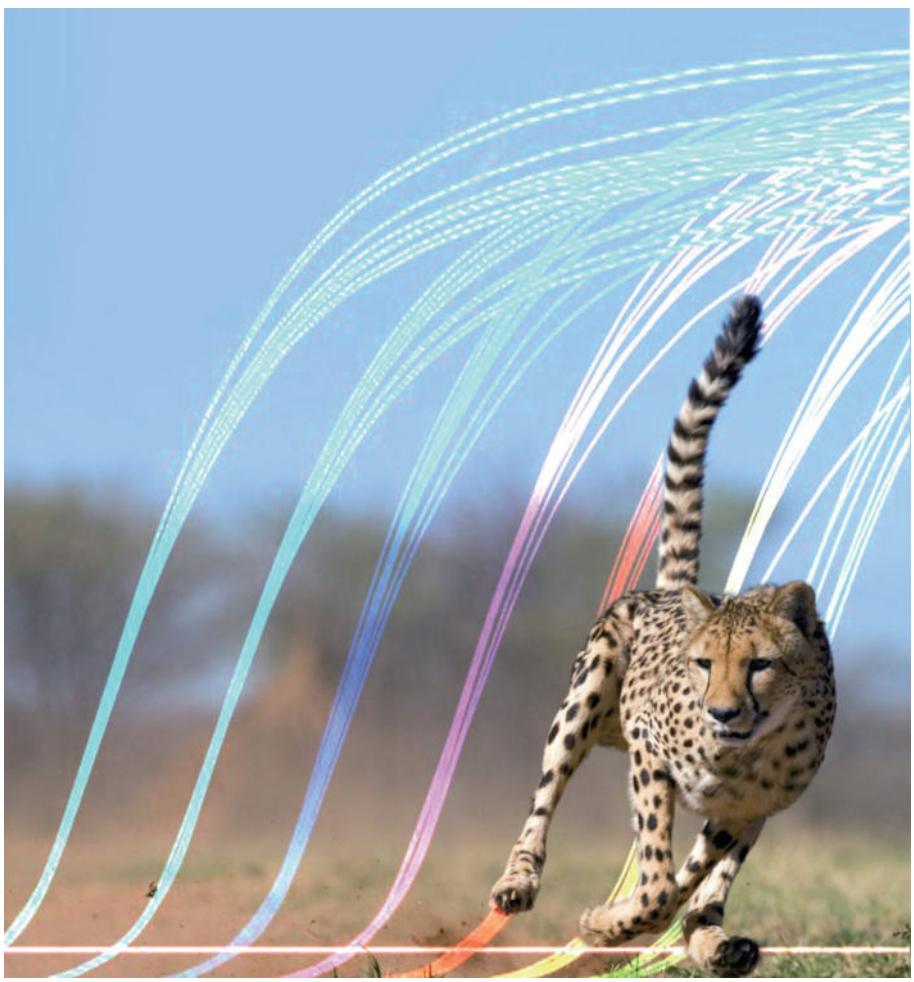
1-Sec Real-time Amplification



Fast Probe Multiplex

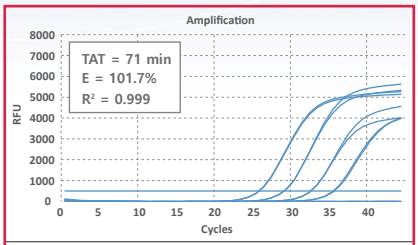
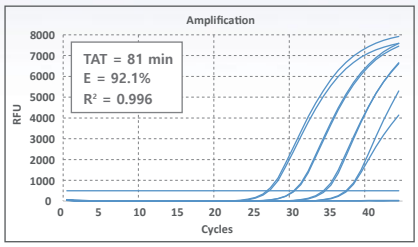
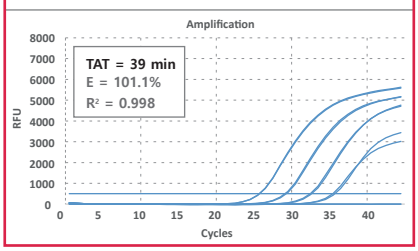
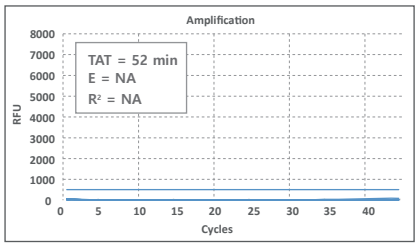
ISO 13485 : 2016

INNOBIZ

NANOHELIX
Reliable Partner for DNA Technologies

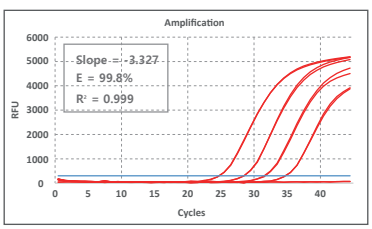
RealHelix™ 1-Sec qPCR Kit

- **Fast:** 30 min for 40 cycles (1-sec denature, 1-sec anneal / extension)
- **Multiplex:** Up to 5-plex probe qPCR in a reaction
- **High sensitivity:** Reliable detection as low as 10 copies of target
- **Robust hot-start enzyme:** Novel Antibody inhibited *Taq* polymerase

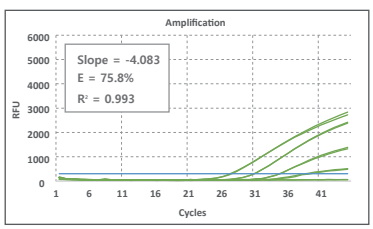
	1-Sec qPCR Kit	General qPCR Kit
Standard Cycle Pre-denature, 5 / 15 min 45 cycles of **95℃, 20 sec** **55℃, 20 sec** 55℃, plate read		
Fast Cycle Pre-denature, 2 / 15 min 45 cycles of **95℃, 1 sec** **55℃, 1 sec** 55℃, plate read		

Comparison of amplification plots of a general qPCR enzyme (RealHelix™ qPCR kit, probe type) and 1-sec qPCR kit using either a standard cycling protocol or a fast cycling protocol. Multiplex Probe qPCR reactions were done with a purified human genomic DNA over range of 0.01 ng to 10 ng per reaction. Each 20 µl reactions were operated on CFX96 real-time PCR detection system (BioRad).

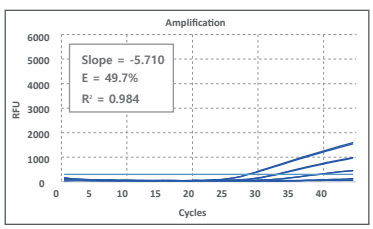
Nanohelix
1-Sec qPCR Kit



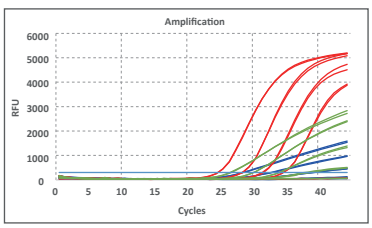
Competitor A
Fast Probe qPCR Kit



Competitor B
Universal Probe supermix (a fast qPCR)



Overlap



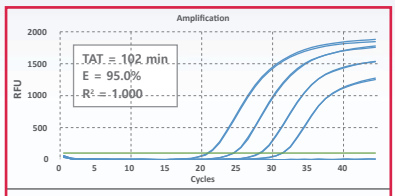
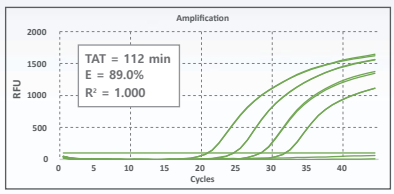
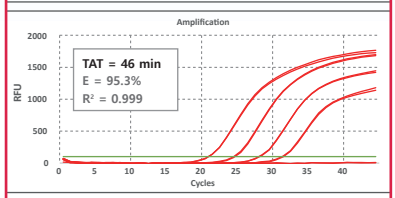
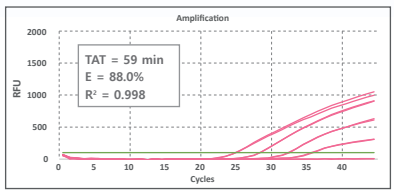
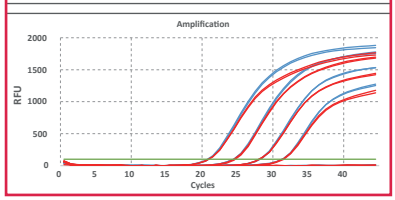
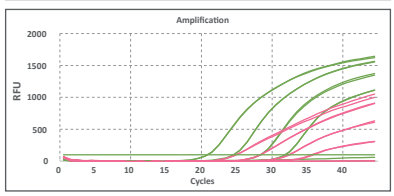
Template (ng)	Nanohelix		Competitor A		Competitor B		⊿avg Ct Nanohelix - A	⊿avg Ct Nanohelix - B
10	25.45	25.49	27.39	27.63	29.50	29.75	- 2.04	- 4.16
	25.47		27.51		29.63			
1	29.08	29.17	31.00	31.06	33.92	34.25	- 1.09	- 4.96
	29.13		31.03		34.09			
0.1	32.24	32.40	34.91	35.08	40.98	41.10	- 2.68	- 8.72
	32.32		35.00		41.04			
0.01	35.44	35.46	39.42	40.17	N/A	N/A	- 4.35	N/A
	35.45		39.80		N/A			

GloB gene fragment was amplified from 10-fold serial dilutions of human genomic DNA (10 ng – 0.1 ng/20 µl reaction) using 1-sec qPCR Kit or other competitor's fast probe qPCR kits: Competitor A (Probe Fast qPCR kit) and Competitor B (Universal Probe supermix) in fast condition. Fluorescence data were obtained in a multiplex (3-plex) qPCR reactions.

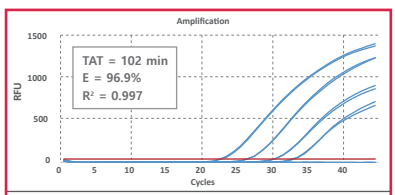
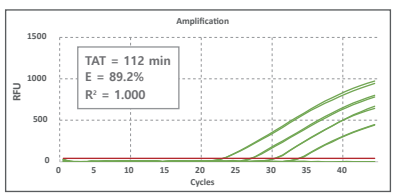
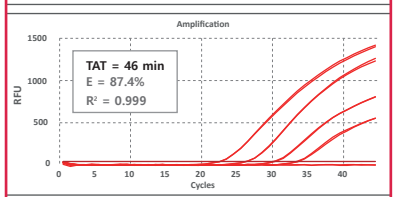
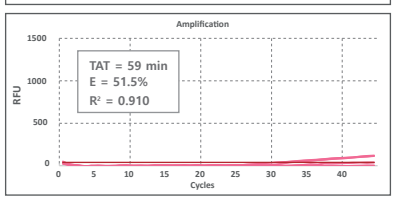
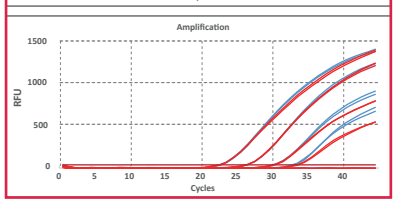
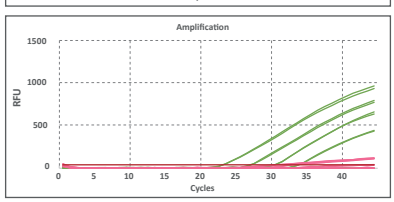
RealHelix™ 1-Sec qRT-PCR Kit

- **Fast:** 40 min for 40 cycles (1-sec denature, 1-sec anneal / extension)
- **Multiplex:** Up to 5-plex probe qRT-PCR in a reaction
- **High sensitivity:** Reliable detection as low as 10 copies of target
- **Robust hot-start enzyme:** Novel Antibody inhibited *Taq* polymerase

H5

	1-Sec qRT-PCR Kit	General qRT-PCR Kit
Standard Cycle RT reaction, 30 min Pre-denature, 5 / 15 min 45 cycles of 95℃, 20 sec 55℃, data acquisition		
Fast Cycle RT reaction, 10 min Pre-denature, 2 / 15 min 45 cycles of 95℃, 1 sec 55℃, 1 sec 55℃, data acquisition		
Fast Cycle **V.S** **Standard Cycle**		

H7

	1-Sec qRT-PCR Kit	General qRT-PCR Kit
Standard Cycle RT reaction, 30 min Pre-denature, 5 / 15 min 45 cycles of 95℃, 20 sec 55℃, 20 sec 55℃, data acquisition		
Fast Cycle RT reaction, 10 min Pre-denature, 2 / 15 min 45 cycles of 95℃, 1 sec 55℃, 1 sec 55℃, data acquisition		
Fast Cycle **V.S** **Standard Cycle**		

Comparison of amplification plots of a general qRT-PCR enzyme (RealHelix™ qRT-PCR kit) and 1-sec qRT-PCR kit using either a standard cycling protocol or a fast cycling protocol. Multiplex Probe qRT-PCR reactions were done with the purified and mixed RNA of Avian influenza type H5 and type H7 (tenfold serial dilutions over range of 1 fg – 1 ng per reaction). Each 20 µl reactions were operated on CFX96 real-time PCR detection system (BioRad).

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NANOHELIX
Reliable Partner for DNA Technologies

Ver. 2105-00

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

Kit contents

RealHelix™ 1-sec qPCR Kit [Probe] w/ UDG (Ver.2.0)		
Cat. No.	**SQPU2-P200 (200 rxns)**	**SQPU2-P500 (500 rxns)**
1-sec 2x Premix [Probe] w/ UDG (V2)	1 ml x 2ea	1 ml x 5ea
ROX Dye (25 μM)	0.2 ml	0.5 ml
Instruction for Use	1 ea	1 ea

Description

RealHelix™ 1-sec qPCR Kit [Probe] w/ UDG (Ver.2.0) 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 kit consists of convenient 2x premix and separate ROX reference dye. The 2x premix contains antibody-inhibited hot-start *Taq* polymerase (*Ab+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

Store -20 ℃

ROX Dye should be stored under dark conditions.

Quality control assay data

Functional analysis

RealHelix™ 1-sec qPCR Kit [Probe] w/ UDG (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.

<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.**

[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 20 µl reaction volume.*

Components	Volumes
DNA Template	**X µl**
1-sec 2x Premix [Probe] w/ UDG (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
SQPU2-P200	RealHelix™ 1-sec qPCR Kit [Probe] w/ UDG (Ver.2.0)	200 rxns
SQPU2-P500	RealHelix™ 1-sec qPCR Kit [Probe] w/ UDG (Ver.2.0)	500 rxns

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