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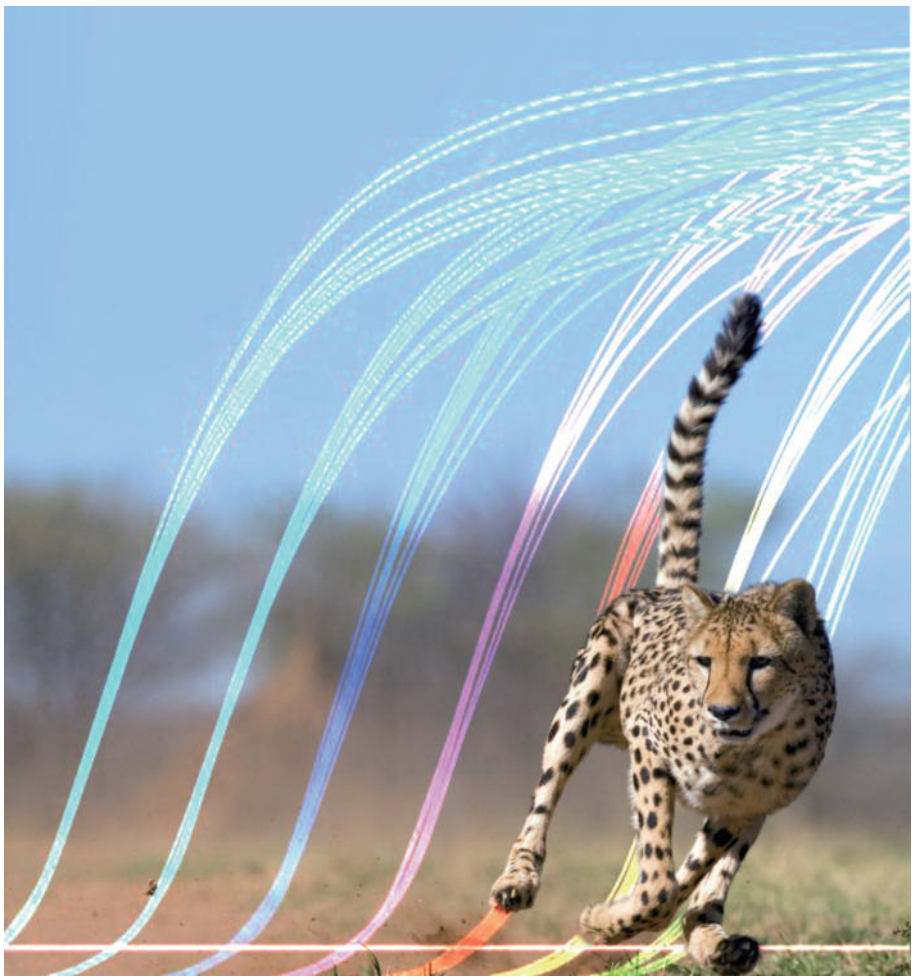
1-Sec Real-time Amplification



Fast Probe Multiplex

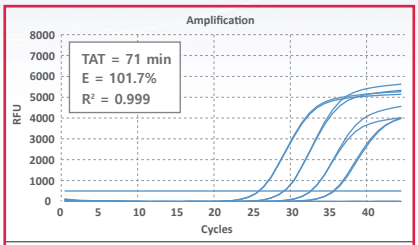
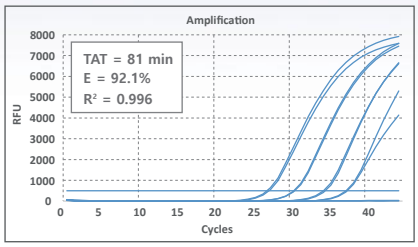
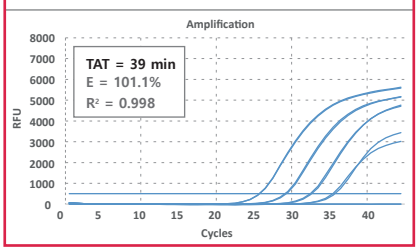
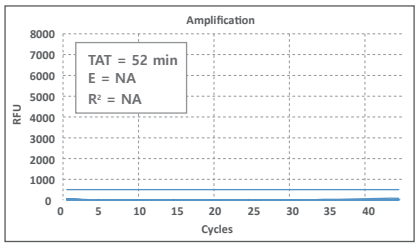
ISO 13485 : 2016

INNOBIZ

NANOHELIX
Reliable Partner for DNA Technologies

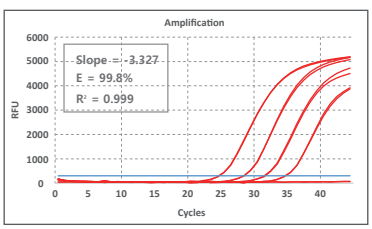
RealHelix™ 1-Sec qPCR Kit

- **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

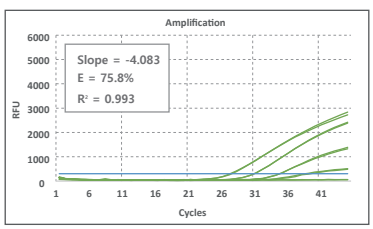
	1-Sec qPCR Kit	General qPCR Kit
Standard Cycle Pre-denature, 5 / 15 min 45 cycles of **95℃, 20 sec** **55℃, 20 sec** 55℃, plate read		
Fast Cycle Pre-denature, 2 / 15 min 45 cycles of **95℃, 1 sec** **55℃, 1 sec** 55℃, plate read		

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).

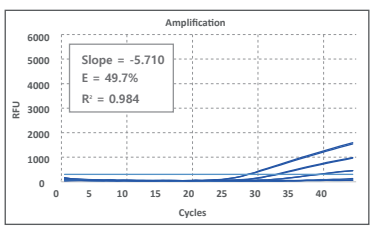
Nanohelix
1-Sec qPCR Kit



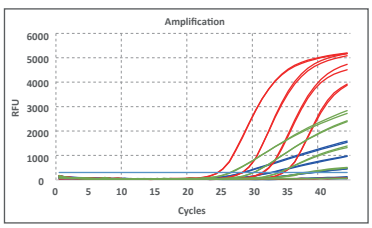
Competitor A
Fast Probe qPCR Kit



Competitor B
Universal Probe supermix (a fast qPCR)



Overlap



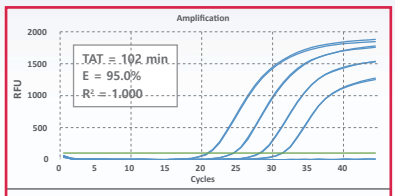
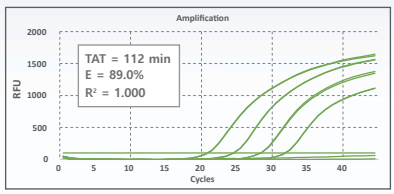
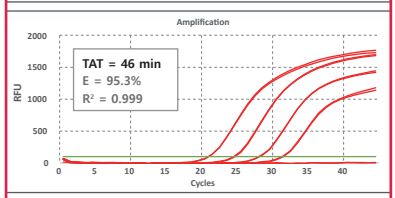
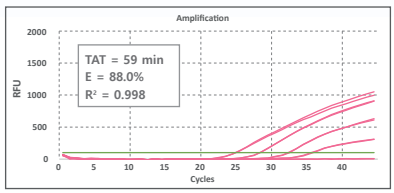
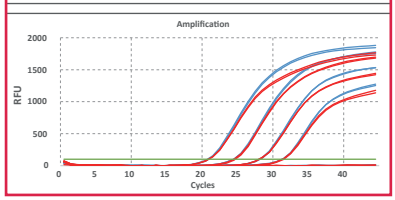
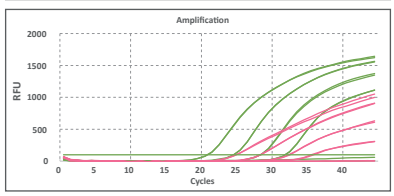
Template (ng)	Nanohelix		Competitor A		Competitor B		⊿avg Ct Nanohelix - A	⊿avg Ct Nanohelix - B
10	25.45	25.49	27.39	27.63	29.50	29.75	- 2.04	- 4.16
	25.47		27.51		29.63			
1	29.08	29.17	31.00	31.06	33.92	34.25	- 1.09	- 4.96
	29.13		31.03		34.09			
0.1	32.24	32.40	34.91	35.08	40.98	41.10	- 2.68	- 8.72
	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.

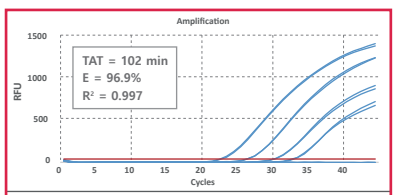
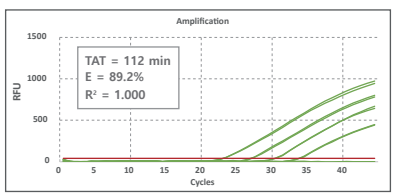
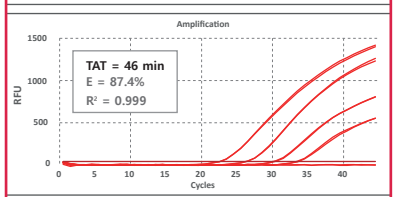
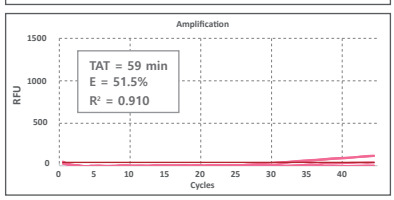
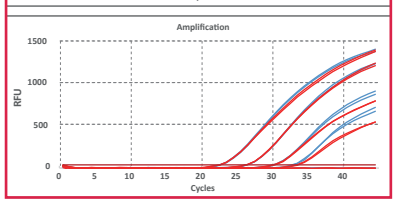
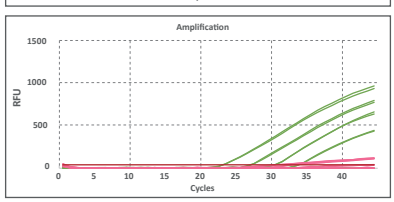
RealHelix™ 1-Sec qRT-PCR Kit

- **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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www.nanohelix.net **info@nanohelix.net**

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CERTIFICATE OF ANALYSIS (1812-V0R0)

RealHelix™ 1-sec qRT-PCR Kit [Probe]

Kit contents

RealHelix™ 1-sec qRT-PCR Kit [Probe]		
Cat. No.	**SQRT-P200 (200 rxns)**	**SQRT-P500 (500 rxns)**
1-sec qRT-PCR 2x Premix [Probe]	1 ml x 2ea	1 ml x 5ea
ROX Dye (25μM)	0.2 ml	0.5 ml
Certificate Analysis	1 ea	1 ea

Description

The RealHelix™ 1-sec qRT-PCR kit [Probe] is designed to perform rapid real-time analysis of RNA samples using fluorescent probe based detection. This kit provides 2x premix and ROX reference dye. The convenient 2x concentrated premix contains an antibody-inhibited hot-start *Taq* polymerase (*Ab+Taq* polymerase), reverse transcriptase, RNase inhibitor, dNTPs, buffers, Mg^{2+}, and 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 within 40-60 min combined with high specificity and sensitivity is achieved with a unique buffer system and balanced enzyme mix.

Application

Quantification of target RNA sample by real-time PCR

Store -20 ℃

ROX Dye should be stored in the dark.

Quality control assay data

Functional analysis

The activity for cDNA synthesis and quantitative PCR of target transcript using RealHelix™ 1-sec

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qRT-PCR Kit was evaluated by real-time PCR using the 10-fold serial-diluted total RNA isolated from human blood and beta-globin gene-specific primer set with ABI 7500 real-time PCR system(Applied Biosystems, USA) and CFX96™ real-time PCR detection system (Bio-Rad, USA).

Quality authorized by: Youn Teak Go 

Protocol

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

<2-step cycling protocol>

Step	Condition		Cycle(s)
cDNA Synthesis		50℃ for 10 ~ 40 min	1
PCR Enzyme activation		95℃ for 2 ~ 5 min	1
PCR Amplification	Denaturation	95℃ for 1~10 sec*	40
	Annealing & extension	60℃ for 1~30 sec* **Collect the fluorescence data**	

<3-step cycling protocol>

Step	Condition		Cycle(s)
cDNA Synthesis		50℃ for 10 ~ 40 min	1
PCR 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 1~20 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℃)

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

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

Components	Volumes
RNA Template	**X µl**
1-sec qRT-PCR 2x Premix [Probe]	**10 µl**
Forward primers (10 µM)	**0.5 ~ 1.0 µl**
Reverse primers (10 µM)	**0.5 ~ 1.0 µl**
Probes (10 µM)	**0.1 ~ 0.5 µl**
ROX Dye (25 µM)	**Optional****
Rnase-free water	**Adjust to final 20 µl**

** 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
SQRT-P200	RealHelix™ 1-sec qRT-PCR Kit [Probe]	200 rxns
SQRT-P500	RealHelix™ 1-sec qRT-PCR Kit [Probe]	500 rxns

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