RealHelix™ *DirectFast* qPCR Kit (V1a)

Kit contents

RealHelix™ *DirectFast* qPCR Kit	
Cat. No.	**DFQPU-A100 (100rxns)**
2x DirectFast qPCR Premix (V1a)	1ml x 1ea
P-Solution	1.5ml x 2ea
10x Dilution Buffer	0.5ml
Instructions for Use	1ea

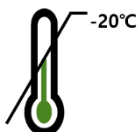
Description

RealHelix™ *DirectFast* qPCR Kit (V1a) is designed for a probe-based rapid qPCR amplification directly from animal tissues, plant tissues, and various clinical samples (including whole blood, serum, urine, hair and swab collections) without any DNA purification processes. The 2x *DirectFast* qPCR Premix contains antibody-inhibited *Taq* DNA polymerase, thermo-labile Uracil-DNA glycosylase, dUTP, dNTPs, $MgCl_2$, a stabilizer, and a unique buffer system to resist various PCR inhibitors of tissue samples. The applied UDG/dUTP system prevents the carryover contamination of PCR products from previous reactions.

Application

Direct and quantitative real-time PCR

Storage



Store below -20℃

Shelf life

12 months

NanoHelix Co., Ltd.

A-dong and B-dong, 43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, Republic of Korea TEL : 82-42-867-9055, FAX : 82-42-867-9057

E-mail : info@nanohelix.net <**www.nanohelix.net** **www.nanohelix.net/KOR**>

Quality Control

By Nanohelix's ISO 13485-certified quality management system, each lot of **RealHelix™ *DirectFast* qPCR Kit (V1a)** was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. SAMPLE PREPARATION

The sample preparation method depends on the type of sample. Please follow the instruction below to prepare the PCR templates.

※ Caution: 1x Dilution Buffer should be freshly prepared before use.

(1) Blood sample (whole blood or serum)

1) Mix 20㎕ of P-Solution with 20㎕ of whole blood or serum samples.
2) Incubate at 90℃ for 10 minutes.
3) Centrifuge at 12,000 rpm for 2 minutes and transfer the supernatant to a new tube.
4) Use 0.1~1㎕ of the supernatant as a PCR template.

(2) Tissue samples (animal and plant tissue)

1) Prepare 1x Dilution Buffer by diluting the provided **10x Dilution Buffer** with PCR-grade water.
2) Take a small piece of tissue (less than 5 mm in diameter) from animal or plant tissue. Plant seeds should be cracked down to a size of less than 1 mm diameter by a small hammer, mortar, bead beater, or tissuelyser.
3) Add 50 ~ 100㎕ of 1x Dilution Buffer to the tissue sample. Briefly mix by tapping or vortexing.
4) Incubate for 3 minutes at room temperature to allow DNA releasing.
5) Centrifuge at 12,000 rpm for 1 minutes and transfer the clear supernatant to a new tube.
6) Use 1~3㎕ of the supernatant solution as a PCR template.

(3) Hair root

1) Prepare 1x Dilution Buffer by diluting the provided **10x Dilution Buffer** with PCR-grade water.
2) Cut off 5 mm size of hair root pieces.
3) Add 50 ~ 100㎕ 1x Dilution Buffer to 1 ~ 3 hair roots. Briefly mix by tapping or vortexing
4) Incubate at room temperature for 3 minutes.
5) Spin down and transfer the solution to a new tube.
6) Use 1~3㎕ of the solution as a PCR template.

(4) Urine

1) Prepare 1x Dilution Buffer by diluting the provided **10x Dilution Buffer** with PCR-grade water.
2) Transfer 1ml of urine into a 1.5ml tube.
3) Centrifuge for 1 minute at 12,000 rpm and remove the supernatant.
4) Suspend the cell pellet in 1ml of 1x PBS buffer (not provided in this kit) and centrifuge for 1 minute at 12,000 rpm to remove supernatant.
5) Add 100㎕ of 1x Dilution Buffer to the cell pellet and briefly mix by tapping or vortexing.
6) Incubate for 3 minutes at room temperature.
7) Centrifuge at 12,000 rpm for 1 minute and transfer the clear supernatant to a new tube.
8) Use 1~3㎕ of suspension as PCR template.

A-dong and B-dong, 43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, Republic of Korea TEL : 82-42-867-9055, FAX : 82-42-867-9057

E-mail : info@nanohelix.net <**www.nanohelix.net www.nanohelix.net/KOR**>

(5) Tissue swabs (any swab samples, including buccal, nasal, vaginal, etc.)

1) Prepare 1x Dilution Buffer by diluting the provided **10x Dilution Buffer** with PCR-grade water.

2) Choose the next steps upon the sample type as followings.

Tissue-collected swab brush

① Put into a 1.5ml tube containing 1ml of 1x PBS (not provided in this kit).

② Suspend the collected tissues by rotating and shaking the swab tip in PBS. Then remove the swab brush from the tube.

Transport medium containing a swab sample

※ Caution: This procedure can only be applied to the specimens collected in UTM, VTM, TE, or equivalent transport medium. The samples should not contain any protein denaturing agents, like guanidine HCl, guanidine thiocyanate, strong detergents, or alcohols.

③ Mix well the tissue suspended medium and transfer 1ml of the medium to a 1.5ml tube.

④ Centrifuge at 12,000 rpm for 1 minute and remove the supernatant.

⑤ Suspend the tissue pellet in 1ml of 1x PBS buffer (not provided in this kit).

3) Centrifuge at 12,000 rpm for 1 minute and remove the supernatant.

4) Add 100㎕ 1x Dilution Buffer to the tissue pellet and briefly mix by tapping or vortexing.

5) Incubate for 3 minutes at room temperature.

6) Centrifuge at 12,000 rpm for 1 minute and transfer the clear supernatant to a new tube.

7) Use 1~3㎕ of solution as a PCR template.

A-dong and B-dong, 43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, Republic of Korea TEL : 82-42-867-9055, FAX : 82-42-867-9057

E-mail : info@nanohelix.net <**www.nanohelix.net www.nanohelix.net/KOR**>

2. PCR CONDITION

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**℃ 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.**

A-dong and B-dong, 43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, Republic of Korea TEL : 82-42-867-9055, FAX : 82-42-867-9057
E-mail : info@nanohelix.net <**www.nanohelix.net www.nanohelix.net/KOR**>

3. REACTION MIX SET-UP

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

Components	Volumes
DNA Template	X µl
2x DirectFast qPCR Premix (V1a)	10µl
Forward primer (10µM)	0.5 ~ 1.0µl
Reverse primer (10µM)	0.5 ~ 1.0µl
Fluorescent Probe (5µM)	0.5 ~ 1.0µl
ROX Dye	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. ROX Dye (Passive Reference) is not included in this kit.

4. Gently mix and briefly centrifuge the reaction mix.

5. Perform the real-time PCR.

Application Note

Products

Cat. No.	Products	Size
DFQPU-A100	RealHelix™ *DirectFast* qPCR Kit (V1a)	100rxns

NanoHelix Co., Ltd.

A-dong and B-dong, 43-15, Techno 5-ro, Yuseong-Gu, Daejeon, 34014, Republic of Korea TEL : 82-42-867-9055, FAX : 82-42-867-9057

E-mail : info@nanohelix.net <www.nanohelix.net www.nanohelix.net/KOR>