# RealHelix™ *DirectFast* qRT-PCR Kit (V1a)

## Kit Contents

| RealHelix™ *DirectFast* qRT-PCR Kit (V1a) | |
|---|---|
| **Cat. No.** | **DFQRU-A100 (100rxns)** |
| 5x DF Reaction Buffer (V1a) | 0.4ml |
| DF Enzyme Mix | 0.2ml |
| 10x Extraction Buffer | 1ml |
| P-Solution | 1.5ml x 2ea |
| Instructions for use | 1ea |

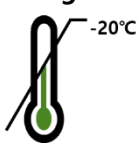
## Description

**RealHelix™ *DirectFast* qRT-PCR Kit (V1a),** a probe-based qRT-PCR kit, is designed for RNA direct real-time amplification from animal tissues, plant tissues, and various clinical samples (including whole blood, serum and swab collections) without any RNA purification processes. This kit allows the fast reaction to complete the qRT-PCR cycles within one hour. The Enzyme Mix in this kit is an optimized blend of Reverse Transcriptase, antibody-coupled *Taq* DNA polymerase, RNase inhibitor, and a heat-labile Uracil-DNA-glycosylase (HL-UDG). The Reaction Buffer contains all of the required components, including optimized buffer components, $Mg^{2+}$, dUTP, and dNTPs. The applied HL-UDG/dUTP system eliminates the carryover contamination of PCR products from previous reactions. HL-UDG efficiently removes uracil residues from dU-containing DNA during the PCR mixture setup and handling.

## Application

Direct and quantitative real-time RT-PCR

**Storage**




Store below -20℃

**Shelf life**

12 months

## Quality Control

By Nanohelix's ISO 13485-certified quality management system, each lot of **RealHelix™ *DirectFast* qRT-PCR 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 samples. Please follow the instructions below to prepare the PCR templates.**

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

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

1) A swab sample collected in TE buffer, PBS buffer, or universal transport medium (UTM, VTM, or UVT)

**※ Caution: do not use other than universal transport medium or equivalents. Buffers or medium containing high salts, strong detergents or chaotropic agents (ex, eNAT) could not be used for this application.**

① Add 10㎕ of 10x Extraction Buffer to 90㎕ of swab collection sample.
② Mix gently by tapping or vortexing and spin down for a few seconds.
③ Use 1~3㎕ of the supernatant as a PCR template.

2) Tissue-collected swab brush

① Prepare 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
③ Centrifuge at 12,000 rpm for 1 min and remove the supernatant.
④ Add 100㎕ 1x Extraction Buffer (prepared by diluting the 10x Extraction Buffer with PCR-grade water) 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 a 1~3㎕ 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) Blood sample** (whole blood or serum)

1) Add 20㎕ of P-Solution to 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 0.5~1㎕ of the supernatant as a PCR template.

**(3) Tissue samples (animal and plant tissue)**

1) Prepare 1x Extraction Buffer by diluting the provided **10x Extraction 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 Extraction Buffer to the tissue sample. Briefly mix by tapping or vortexing.
4) Incubate for 3 minutes at room temperature to allow RNA releasing.
5) Centrifuge at 12,000 rpm for 1 minute and transfer the clear supernatant to a new tube.
6) Use 1~3㎕ of the supernatant solution as a PCR template.

## 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℃, thermocycling can be performed using a 2-step cycling protocol as follow.

| Step | Condition | | Cycle(s) |
|---|---|---|---|
| [Optional] UDG reaction* | | 20 ~ 25℃ for 5 min | 1 |
| cDNA Synthesis | | 50℃ for 10 min | 1 |
| PCR Enzyme Activation | | 95℃ for 2 ~ 5 min | 1 |
| PCR Amplification | Denaturation | 95℃ for 1 ~ 10 sec* | 40 |
| | Annealing & Extension | **AT**℃ 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-step cycling protocol>**

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

| Step | Condition | | Cycle(s) |
|---|---|---|---|
| [Optional] UDG reaction* | | 20 ~ 25℃ for 5 min | 1 |
| cDNA Synthesis | | 50℃ for 10 min | 1 |
| Enzyme Activation | | 95℃ for 2 ~ 5 min | 1 |
| PCR Amplification | Denaturation | 95℃ for 1 ~ 10 sec* | 40 |
| | 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.**

## 3. REACTION MIX SETUP

**Add the following components in a real-time PCR tube for a single 20µl reaction.***

| Components | Volumes |
|---|---|
| Template | 1.0 ~3.0ul |
| 5x DF Reaction Buffer (V1a) | 4.0ul |
| DF Enzyme Mix | 2.0ul |
| Forward Primer (10µM) | 1.0ul |
| Reverse Primer (10µM) | 1.0ul |
| Probe (10µM) | 0.5ul |
| ROX Dye | Optional ** |
| RNase-free Water | X ul |
| Total volume per reaction | 20ul |

* For multiple reactions, prepare a master mix by adding the required volumes of each above components (except the template) and dispense appropriate volumes into each PCR tubes or wells in a plate.



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>**

The reaction volume for a reaction could be adjusted according to the manufacturer's instructions of the instruments.

** 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 qRT-PCR.**

**Application Note**

## Products

| Cat. No. | Products | Size |
|---|---|---|
| **DFQRU-A100** | RealHelix™ *DirectFast* qRT-PCR Kit (V1a) | 100rxns |



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**>