Ver. 2208-01

HelixAmp™ Direct RT-PCR Kit

Kit Contents

HelixAmp™ Direct RT-PCR Kit		
Cat. No.	**DRT200** (200rxn)	**DRTU200** (200rxns)
Enzyme Mix [DRT]	0.4ml x 1ea	-
Enzyme Mix [DRTU]	-	0.4ml x 1ea
2x Buffer Mix [DRT]	1.25ml x 4ea	-
2x Buffer Mix [DRTU]	-	1.25ml x 4ea
10x Dilution Buffer	1.5ml x 2ea	1.5ml x 2ea
6x Loading Dye	1ml x 1ea	1ml x 1ea
Instruction for Use	1ea	1ea

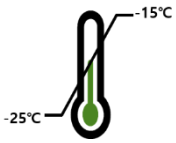
Description

HelixAmp™ Direct RT-PCR Kit is designed for the amplification and detection of target RNA directly from whole blood, animal tissue, and plant tissues without any RNA purification processes. This kit contains enzyme mixture including HelixCript™ *Thermo* Reverse Transcriptase and antibody-coupled *Taq* polymerase, and 2x Buffer Mix containing dNTPs, $MgCl_2$, and unique buffer system to resist various PCR inhibitors from tissue samples. A Uracil-DNA glycosylase and dUTP applied version is also available.

Application

- Point-of Care Molecular diagnostics
- Direct amplification of target RNA from samples
- Direct detection of RNA viral pathogens from various tissues

Storage



-20±5℃

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.co.kr/KOR>

Quality Control

In accordance with NanoHelix's ISO 13485-certified Quality Management System, each lot of **HelixAmp™ Direct RT-PCR Kit** was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. Sample Preparation

[Whole Blood or Saliva]

1) Directly add **1-5µl of sample** to RT-PCR reaction mix without any pre-treatment.
   ※ **Heparin, EDTA or citrate-treated whole blood are all suitable for this kit.**

[Swab sample]

1) Make 1x Dilution Buffer by diluting the 10x Dilution Buffer with distilled water.

2) Place the swab brush into a 1.5ml microcentrifuge tube containing the **300µl of PBS** (Phosphate Buffer Saline. Not included in this kit) and **rotate the brush to the 5-10times.** Press the brush against the side of the tube and rotate the brush. After removing it from the tube, the liquid remains in the tube.

3) Centrifuge at **12,000rpm for 3min** at room temperature. Discard the supernatant.

4) Add the 100µl of 1x Dilution Buffer into the tube including the harvested sample.

5) Briefly mix by tapping or vortexing.
   ※ **Make sure that the samples are soaked in the Dilution Buffer.**

6) Incubate at RT for 3min for tissue lysis and RNA releasing.

7) Centrifuge briefly and transfer 1-5µl of the lysate supernatant into a RT-PCR reaction mix prepared as followings.
   ※ **The lysate supernatant should be removed from the tissues and kept at -20℃ for next uses. The stored lysate could be used for several weeks.**

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.co.kr/KOR>**

[Animal or Plant tissue]

1) Make 1x Dilution Buffer by diluting the 10x Dilution Buffer with RNase-free Water.

2) Take a small piece of tissue from animal or plant tissue **(Do not exceed 6mm)**. Plant seeds should be cracked down to less than 1mm diameter size by a small hammer, bead beater, or tissuelyser.
   ※ **To prevent cross-contaminations, recommend to use disposable cutting tools. In case of non-disposable cutting tool, make sure the cutting tools were properly cleaned with 2% sodium hypochlorite.**

3) Add tissue sample into the 1x Dilution Buffer as follows according to the sample size.

Sample size (diameter)	1~2㎜	3~4㎜	5~6㎜
Volume of Dilution buffer	50㎕	100㎕	150㎕

4) Briefly mix by tapping or vortexing.
   ※ **Make sure that the samples are soaked in the dilution buffer.**

5) Incubate at RT for 3min for tissue lysis and RNA releasing.

6) Centrifuge briefly and transfer 1-5μl of the lysate supernatant into a RT-PCR reaction mix prepared as followings.
   ※ **The lysate supernatant should be removed from the tissues and kept at -20℃ for next uses. The stored lysate could be used for several weeks.**

2. Add the following components into a 0.2ml micro-tube and mix well.

Components	Volume
Sample prepared	1~5μl
Enzyme Mix	2μl
2x Buffer Mix	25μl
Forward Primer (10pmoles/μl)	2μl
Reverse Primer (10pmoles/μl)	2μl
RNase-free Water	Up to 50μl

3. Program the thermal cycler as follows for direct RT-PCR.

Step	Temperature/time	Cycles
[1] **[Optional] UDG Reaction** This step is only required for the UDG included product.	20~25℃, 5 min	x 1
Reverse transcription	50℃, 30 min	x 1
Pre-denaturation	95℃, 5 min	x 1
Denaturation	95℃, 20 sec	x 40~45
Primer annealing	[2] X℃, 30-45 sec	
Extension	72℃, 1 min/kb	
Post Extension	72℃, 5 min	x 1

[1] **The UDG reaction step is not essential. The UDG will efficiently remove carryover contaminant DNA during sample setup and cycler ramping.**

[2] **Annealing Temperature = T_m – (4 ~ 6℃)**

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

4. Spin-down each tube, and perform the RT-PCR.

Important Note

- In case of blood sample, aggregates of blood debris and proteins will be appeared after PCR. Spin the reactions at 600 x g (about 3,000rpm) for 1minutes to pellet the debris.

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.co.kr/KOR>

Trouble shooting

No Product or low yield

- Make sure the pipetting and cycling protocols were performed as recommended.
- Decrease template amount or dilute the supernatant (1:5 or 1:10)
- **Optimize annealing temperature.** Perform a temperature gradient PCR.
- **Increase** the number of **PCR cycles**.
- **Check primers** or design new primers.

Non-specific product

- Make sure the extension time used was not too long (>1min/kb).
- **Decrease template** amount or **dilute** the supernatant (1:5 or 1:10)
- **Optimize annealing temperature.** Perform a temperature gradient PCR.
- Decrease primer annealing time.
- Decrease primer concentration.
- **Reduce** the number of **PCR cycles**.
- **Check primers** or design new primers.

Cross-contamination

For prevention of PCR contamination

- Use disposable pipet tips containing hydrophobic filters.
- Use a separate aliquot of D.W stock for each round of PCR.
- Make sure that work space and instruments are decontaminated at regular intervals.

Products

Cat. No.	Products	Size
DRT200	HelixAmp™ Direct RT-PCR Kit	200rxns
DRTU200	HelixAmp™ Direct RT-PCR Kit [UDG System]	200rxns

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.co.kr/KOR>**