Ver. 2208-01

HelixAmp™ Direct PCR [3G]

Kit Contents

HelixAmp™ Direct PCR [3G]		
Cat. No.	**DPR200** (200rxns)	**DPRU200** (200rxns)
2x Direct PCR Premix	1ml x 5ea	-
2x Direct PCR Premix [UDG System]	-	1ml x 5ea
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 PCR [3G] is designed for the PCR amplification directly from animal tissues, whole blood, and plant tissues without any DNA purification processes. This kit contains a 2x reaction mix including *Taq* DNA polymerase, dNTPs, $MgCl_2$, and unique buffer system to resist various PCR inhibitors of tissue samples. Unlike other brands of direct PCR which based on a derivative of *Pfu* DNA polymerase, NanoHelix's Direct PCR [3G] is based on *Taq* polymerase. Due to this enzyme's robust amplification and 3′ to 5′ exo-negative properties, this kit could be used for allele specific PCR which is routinely used for various genotyping. Moreover the Uracil-DNA glycosylase with dUTP system for the prevention of carryover contamination can be applied to this *Taq* polymerase-based kit. dUTP could not be used with the *Pfu* DNA polymerase and its derivatives. A Uracil-DNA glycosylase and dUTP applied version is also available.

Application

- Direct PCR amplification of target DNA without any DNA purification from various sample types such as whole blood, saliva, mouse tissues (tail, heart, liver, large intestine, small intestine, kidney, stomach, ear, brain, spleen), zebrafish fin, pork, beef, plant tissues (leaf and seed).
- Allele-specific PCR.
- PCR for genotyping
- PCR for selection of genetically modified organisms (GMO).

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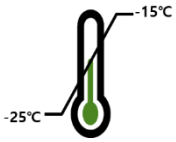
Storage



-20±5℃

Shelf life

12 months

Quality Control

In accordance with NanoHelix's ISO 13485-certified Quality Management System, each lot of **HelixAmp™ Direct PCR [3G]** was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. Sample Preparation

[Whole Blood or saliva]

1) Directly add **1-3µl of sample** to PCR reaction mix without any pre-treatment.
   ※ **Heparin, EDTA or citrate-treated whole blood are all suitable for this kit.**

[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 DNA releasing.

6) Centrifuge briefly and transfer 1-3μl of the lysate supernatant into a PCR reaction mix prepared as followings.

※ **The lysate supernatant should be removed from the tissues and keep 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	1~3μl
2x Direct PCR Premix	25μl
Forward Primer (10pmoles/ul)	2μl
Reverse Primer (10pmoles/ul)	2μl
RNase-free Water	Up to 50μl

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

Step	Temperature/time	Cycles
[1] **[Optional] UDG reaction** This step is only required for the UDG included product.	50℃, 5 min	x 1
Pre-denaturation	95℃, 5 min	x 1
Denaturation	95℃, 20 sec	x 35 ~40
Primer annealing	[2] X℃, 30 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 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.

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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.
- To prevent carryover contamination, using of UDG applied kit (**Catalog No. DPRU200**) is recommended.

Products

Cat. No.	Products	Size
DPR200	HelixAmp™ Direct PCR [3G]	200rxns
DPRU200	HelixAmp™ Direct PCR [3G] [UDG System]	200rxns

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