Ver. 1903-00

HelixAmp™ Direct PCR [3G] (Hot-start)

Kit contents

HelixAmp™ Direct PCR [3G] (Hot-start)		
Cat. No.	**DPRH200 (200 rxns/kit)**	**DPRHU200 (200 rxns/kit)**
2x Direct PCR premix [Hot-start]	1 ml x 5 ea	–
2x Direct PCR premix [Hot-start] (with UDG)	–	1 ml x 5 ea
10x Dilution Buffer	1.5 ml x 2 ea	1.5 ml x 2 ea
6x Loading Dye	1 ml x 1 ea	1 ml x 1 ea
Certificate Analysis	1 ea	1 ea

*** Store at -20℃**

Description

HelixAmp™ Direct PCR [3G] (Hot-start) 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 Hot-start *Taq* DNA polymerase, dNTPs, $MgCl_2$, and unique buffer system to resist various PCR inhibitors of tissue samples. Hot-start *Taq* DNA polymerase is inactive at lower temperature by an inhibitory antibody and using this enzyme avoids the polymerization from non-specifically bound primers during the setting of PCR mix and at the start of PCR cycles. At high temperature of the initial denaturation step of PCR, the inhibitory antibody is released by denaturation and the free *Taq* DNA polymerase becomes active. 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).

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- Allele-specific PCR.
- PCR for genotyping
- PCR for selection of genetically modified organisms (GMO).

Quality control assay data

HelixAmp™ Direct PCR [3G] (Hot-start) is evaluated by amplification of PCR product corresponding to each sample type-specific DNA region directly using whole blood, plant leaf tissue, or animal tissue according to protocol.

Quality authorized by Yountaek Go 

Protocol

1. Sample Preparation

[Whole Blood or saliva]

1) Directly add **1-3 µl 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 distilled water.

2) Take a small piece of tissue from animal or plant tissue **(Do not exceed 6 mm)**. Plant seeds should be cracked down to less than 1 mm 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 3 min for tissue lysis and DNA releasing.

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6) Centrifuge briefly and transfer 1-3 µl 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 0.2 ml micro-tube and mix well.

Components	Volume
Sample prepared	1~3 µl
2x Direct PCR premix	25 µl
Forward Primer (10 pmoles/ul)	2 µl
Reverse Primer (10 pmoles/ul)	2 µl
D.W.	Up to 50 µl

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

Step	Temperature/time	Cycles
[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	X℃ , 30 sec	
Extension	72℃, 1 min/kb	
Post Extension	72℃, 5 min	x 1

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 3 000 rpm) for 1 minutes 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 (>1 min/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 (**DPRHU200**) is recommended.

Products

Cat. No.	Products	Size
DPRH200	HelixAmp™ Direct PCR [3G] (Hot-start)	200 rxns
DPRHU200	HelixAmp™ Direct PCR [3G] (Hot-start) (Containing UDG/dUTP)	200 rxns

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