RealHelix™ Direct qPCR Kit [Green]

Kit Contents

RealHelix™ Direct qPCR Kit [Green]		
Cat. No.	**DQPR-S200 (200rxns)**	**DQPR-S500 (500rxns)**
2x Direct qPCR Premix [Green]	1.25ml x 2ea	1.25ml x 5ea
P-Solution	1.5ml x 4ea	1.5ml x 10ea
10x Dilution Buffer	1ml	1.25ml x 2ea
Instructions for Use	1ea	1ea

Description

RealHelix™ Direct qPCR Kit [Green] is designed for an intercalating dye based 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 Direct qPCR Premix [Green] in this kit contains antibody-inhibited *Taq* DNA polymerase, dNTPs, $MgCl_2$, SYBR Green, stabilizer, and unique buffer system to resist various PCR inhibitors of tissue samples.

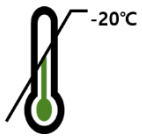
Application

Direct and quantitative real-time PCR

Store

2x Direct qPCR Premix [Green] should be stored in a dark.

Storage



Store below -20℃

Shelf life

12 months

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Quality Control

By Nanohelix's ISO 13485-certified quality management system, each lot of **RealHelix™ Direct qPCR Kit [Green]** 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 1~3㎕ 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.

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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 1x Dilution Buffer 100㎕ 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.

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.

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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

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 min 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 min 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㎕ solution as a PCR template.

2. Program a real-time PCR instrument according to the recommendations below.

Step	Condition		Cycle(s)
Enzyme Activation		95℃ for 5 min	1
PCR Amplification	Denaturation	95℃ for 20 sec	40
	Annealing	[1)]**AT**℃ for 20~30 sec	
	Extension	72℃ for 1 min/kb **Collect the fluorescence data**	

[1)] **AT : annealing temperature**

Annealing Temperature = T_m – (4 ~ 6℃)

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

3. Add the following components in a PCR tube for a single 25µl reaction.*

Components	Volumes
DNA Template	1~3µl
2x Direct qPCR Premix [Green]	12.5µl
Forward primer (5µM)	0.5 ~ 1.0µl
Reverse primer (5µM)	0.5 ~ 1.0µl
ROX Passive Reference Dye	Optional **
RNase-free Water	Adjust to final 25µl

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

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

** Use the recommended amount or concentration of ROX Passive Reference Dye depending on the instrument.

4. Gently mix and briefly centrifuge the reaction mix.

5. Perform the real-time PCR.

Products

Cat. No.	Products	Size
DQPR-S200	RealHelix™ Direct qPCR Kit [Green]	200rxns
DQPR-S500	RealHelix™ Direct qPCR Kit [Green]	500rxns

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