

RealHelix™ Premier qPCR Kit [Green, High ROX]

Kit Contents

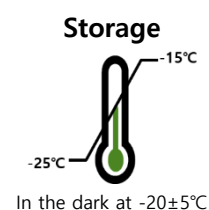
RealHelix™ Premier qPCR Kit [Green, High ROX]		
Cat. No.	PQH-S200 (200rxns)	PQH-S500 (500rxns)
Premier 2x Premix [GH]	1ml x 2ea	1ml x 5ea
Instruction for Use	1ea	1ea

Description

The RealHelix™ Premier qPCR Kit [Green, High ROX] is designed to perform a rapid, highly specific, and sensitive real-time quantification of target DNA. Specially designed buffer and antibody-mediated hot start enzyme considerably reduce the primer dimer formations and ensure producing reliable data. The convenient 2x concentrated premix contains *Ab+Taq* polymerase, dNTPs, buffers, Mg²⁺, a green fluorescent dye, ROX passive dye (1μM concentration), and stabilizing agent. The ROX dye does not take part in the PCR reaction but allows to normalize for non-PCR related signal variation and provides a baseline in multiple reactions.

Application

Quantification of target DNA sample by real-time PCR



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Quality Control

In accordance with NanoHelix's ISO 13485-certified Quality Management System, each lot of RealHelix™ Premier qPCR Kit [Green, High ROX] was tested against predetermined specifications to ensure consistent product quality.

Protocol

1. Program a real-time PCR instrument according to the recommendations below. The excitation and emission maxima of the included green fluorescence dye are at 494 nm and 521 nm, respectively.

<2-step cycling method >

Step	Condition		Cycle(s)
Enzyme Activation		95°C for 5 min	1
PCR Amplification	Denaturation	95°C for 20 sec	40
	Annealing & Extension	60°C for 40 sec Collect the fluorescence data	

<3-step cycling method >

Step	Condition		Cycle(s)
Enzyme Activation		95°C for 5 min	1
PCR Amplification	Denaturation	95°C for 20 sec	40
	Annealing	¹⁾ AT°C for 30 sec	
	Extension	72°C for 30 sec Collect the fluorescence data	

¹⁾ AT, annealing temperature of primers used

$$\text{Annealing Temperature} = T_m - (4 \sim 6^\circ\text{C})$$

$$\text{Where, } T_m (\text{Melting Temp.}) = [4^\circ\text{C} \times (\text{G} + \text{C})] + [2^\circ\text{C} \times (\text{A} + \text{T})]$$

☞ For melting curve analysis of products, refer to the instrument documentation

2. Add following components for a 20 μ l volume reaction.

Components	Volumes
DNA Template	X μ l
Premier 2x Premix [GH]	10 μ l
Forward primers (10 μ M)	0.5 μ l (final 0.25 μ M) ¹⁾
Reverse primers (10 μ M)	0.5 μ l (final 0.25 μ M) ¹⁾
RNase-free Water	Adjust to final 20 μ l

¹⁾ The amount of each primer should be adjusted according to the efficiencies of target amplification.

3. Gently mix and briefly centrifuge the reaction mix.

4. Perform the Real-time PCR.

※ Instrument compatibility

- 7900HT Fast Real-Time PCR System (Applied Biosystems)
- 7300 Real-Time PCR System (Applied Biosystems)
- 5700 Real-Time PCR System (Applied Biosystems)
- StepOnePlus™ Real-Time PCR System (Applied Biosystems)
- StepOne™ Real-Time PCR System (Applied Biosystems)

Products

Cat. No.	Products	Size
PQH-S200	RealHelix™ Premier qPCR Kit [Green, High ROX]	200rxns
PQH-S500	RealHelix™ Premier qPCR Kit [Green, High ROX]	500rxns

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