# RealHelix™ qPCR Kit [Green]

## Kit Contents

| RealHelix™ qPCR Kit [Green] | | |
|---|---|---|
| **Cat. No.** | **QP2-S200 (200rxns)** | **QP2-S500 (500rxns)** |
| 2x Premix [Green] | 1ml x 2ea | 1ml x 5ea |
| ROX Dye (25μM) | 0.2ml | 0.5ml |
| Instructions for Use | 1ea | 1ea |

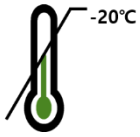
## Description

**RealHelix™ qPCR Kit [Green]** is designed to perform a rapid real-time quantification of target DNA using a double strand DNA-binding green fluorescent dye. The convenient 2x premix contains hot-start PCR enzyme, green fluorescent dye, dNTPs, buffers, $Mg^{2+}$, and stabilizing agent. The hot-start PCR enzyme provides high specific amplification of target DNA and minimizes the side products such as primer dimers. The separately supplied ROX Dye could be used as a passive reference dye in real-time PCR instruments that are compatible with the evaluation of the ROX signal.

## Application

Quantification of target DNA

**Storage**




In the dark at -20℃.

**Shelf life**

12 months



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## Quality Control

By Nanohelix's ISO 13485-certified quality management system, each lot of **RealHelix™ qPCR Kit [Green]** 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℃ for 15 min | 1 |
| PCR Amplification | Denaturation | 95℃ for 20 sec | 40 |
| | Annealing & Extension | 60℃ for 40 sec<br>**Collect the fluorescence data** | |

**<3-step cycling method>**

| Step | Condition | | Cycle(s) |
|---|---|---|---|
| Enzyme Activation | | 95℃ for 15 min | 1 |
| PCR Amplification | Denaturation | 95℃ for 20 sec | 40 |
| | Annealing | [1]**AT**℃ for 30 sec | |
| | Extension | 72℃ for 1 min/kb<br>**Collect the fluorescence data** | |

[1] **AT,** annealing temperature of primers used

Annealing Temperature = $T_m$ − (6 ~ 8℃)

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

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


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**2. Add following components for a 20µl volume reaction.**

| Components | Volumes |
|---|---|
| DNA Template | X µl |
| 2x Premix [Green] | 10µl |
| Forward primers (10µM) | 0.5µl (final 0.25µM)[1)] |
| Reverse primers (10µM) | 0.5µl (final 0.25µM)[1)] |
| ROX Dye (25µM) | Optional [2)] |
| RNase-free Water | Adjust to final 20µl |

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

[2)] Use the recommended amount or concentration of ROX Passive Reference Dye depending on the instrument.

**3. Gently mix and immediately centrifuge the reaction mix.**

**4. Perform the Real-time PCR.**

## Products

| Cat. No. | Products | Size |
|---|---|---|
| **QP2-S200** | RealHelix™ qPCR Kit [Green] | 200rxns |
| **QP2-S500** | RealHelix™ qPCR Kit [Green] | 500rxns |



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