Novel Express qPCR System-ROX Free

09 MAR 2021

Catalog Number	Size	Concentration
QP003-0100	100 reactions (20 µl vol)	2X

Storage Conditions

Stable for up to 1 year at -20°C

Description

The Novel Express qPCR System-ROX Free provides a convenient, simple, rapid, high sensitivity, specificity, stability, and robust set-up for performing quantitative real-time analysis of DNA samples. The kit is with the proprietary concentration with the HotStart DNA polymerase、SYBR Green I、dNTPs、Mg^{2+}、 reaction buffer, and enhance stabilizer. The HotStart DNA polymerase is inactive under the 75°C that avoid the non-specific hybrid or primer dimer at the room temperature.

The SYBR Green I fluorescent dye was specific binding with the DNA double strands ensure the consistency of the fluorescent signal and PCR Product.

Novel Express qPCR System- ROX Free is supplied at the 2X concentration to allow approximately 50% of the final reaction volume to be used for the addition of primer, template solutions, and RNase–free H_2O. Reagents are provided with the sufficient amplification reactions of 20 µl each.

Kit Content(s)

Novel Express qPCR Reagent	1000 µl

Required materials but not provided

- A compatible real-time PCR instrument
- Vortex or equivalent
- Microcentrifuge
- Plates and seals for your instruments

Instrument Compatibility

Product Name	Real-time PCR Instrument
Novel Express qPCR System-ROX Free	Thermal Cycler Dice real time system, LightCycler, Smart cycler system · Corbett rotor-gene 6000

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Reaction Setup

1. PCR reaction, assemble the following components in a 0.2 ml PCR tube on ice just prior to use.

Component	Volume (µl)	Final Concentration
DNA Sample	0.4	10~100 ng DNA or 1~10 ng cDNA
Forward Primer (10 µM)	0.4	0.1~1 µM
Reverse Primer (10 µM)	0.4	0.1~1 µM
Novel Express qPCR Reagent	10	1X
RNase–Free H_2O	To 20	--

2. Mix gently. If necessary, centrifuge briefly. Cap tubes and place in the thermal cycler.
3. Process in the thermal cycler for 35~45 cycles as follows:

Initial Denaturation	2 minutes at 95°C (5 min for GC rich or complicated template)
Denaturation	15 seconds at 95°C
Annealing	15-30 seconds at 55~65°C
Extension	35 seconds at 72°C back to denaturation 35~45cycle
Melting curve	Refer to specific guidelines for instrument used

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

Template

Purified high quality DNA is needed for a success PCR reaction. The final concentration of cDNA template please refer to "Reaction Setup".

Important notes

1. Shake gently before use to avoid foaming and low-speed centrifugation.
2. Reduce the exposure time.
3. This product is not available for hybridization probe method.
4. The ROX reference dye keeps away from light.
5. During operation, always wear a lab coat, disposable gloves, and protective equipment.

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Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantify of nucleic acid targets with the kit.

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Material	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
	Inadequate Thermal Cycling Conditions	1. Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 10% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Primer Design	1. Verify primers design at different annealing temperatures.
Low or High Reaction Efficiency	Primer- Dimer	1. Reduce primer concentration. 2. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary. 3. Perform melt-curve analysis to determine if primer- dimers are present.
	Insufficient Optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.

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