PRODUCT INFORMATION SHEET

Q4ever™ Green *2000X DMSO Solution*

Catalog number: 17608, 17609
Unit size: 50 ul, 1 ml

Component	Storage	Amount (Cat No. 17608)	Amount (Cat No. 17609)
Q4ever™ Green *2000X DMSO Solution*	Freeze (< -15 °C), Minimize light exposure	50 µL	1 mL

OVERVIEW

A real-time polymerase chain reaction (real-time PCR), also known as quantitative polymerase chain reaction (qPCR), is a popular laboratory technique of molecular biology based on the polymerase chain reaction (PCR). It monitors the amplification of a targeted DNA molecule during the PCR (i.e., in real time), not at its end, as in conventional PCR. Real-time PCR can be used quantitatively (quantitative real-time PCR) and semi-quantitatively (i.e., above/below a certain amount of DNA molecules) (semi-quantitative real-time PCR). There are two common methods for the detection of PCR products in real-time PCR, including (1) non-specific fluorescent dyes that bind any double-stranded DNA; and (2) sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter, which permits detection only after hybridization of the probe with its complementary sequence. For the first method, there are two requirements for a DNA binding dye for real-time detection of PCR, i.e., (a). enhanced fluorescence when bound to double-stranded DNA; and (b). minimal inhibition of PCR. SYBR Green is predominantly used in a variety of qPCR applications. We have recently developed Q4ever™Green, a newer generation of SYBR Green, to address some limitations with SYBR Green, e.g., enzyme inhibition. Q4ever™Green permits the use of the Q4ever™Green in PCR with little PCR inhibition and enhanced sensitivity. Q4ever™Green can be used to monitor the amplification of any double-stranded DNA sequence. No probe is required, which can reduce assay setup and running costs, assuming that your PCR primers are well designed and your reaction is well characterized. As SYBR Green, the primary disadvantage is that it may generate false positive signals; i.e., because the Q4ever™Green dye binds to any double-stranded DNA. It can also bind to nonspecific double-stranded DNA sequences. It is extremely important to have well-designed primers that do not amplify non-target sequences, and that melt curve analysis be performed.

AT A GLANCE

Storage and Handling
Store at -20 °C, protected from light. Product is stable for at least 12 months from the date of receipt when stored as recommended.

Safety
We advise researchers to follow universal laboratory safety precautions when handling Q4ever Green dye.

PREPARATION OF WORKING SOLUTION

Q4ever Green working solution (50X)
Dilute the 2000X Q4ever Green stock solution to make a 50X Q4ever Green stock solution using water or TE buffer.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol is recommended. Adjust the protocol if needed to achieve optimal results.

Set up the PCR reaction as follows:
5 µL of 10X polymerase buffer without magnesium
2.5 µL of 50 mM $MgCl_2$
2 µL of 50X Q4ever Green working solution
2 µL of 5 mM dUTP
1-5 units of DNA polymerase
Desired amount of cDNA
100-1000 nM each of primers (Final concentrations of Forward and Reverse primers)
Adjust the final volume to 50 µL with dH_2O

Perform real-time PCR
Perform real-time PCR on a thermocycling fluorometer and record the fluorescence signal.

EXAMPLE DATA ANALYSIS AND FIGURES

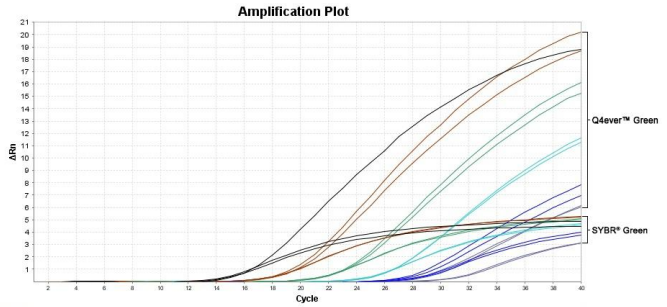

Figure 1. A comparison of the relative fluorescence signal from qPCR reactions performed with Q4ever ™ Green and SYBR ™ Green. Q4ever ™ Green has much brighter signal than SYBR ™ Green.

DISCLAIMER

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