BIO-RAD

iScript™ One-Step RT-PCR Kit With SYBR® Green

170-8892 50 x 50 µl reactions
170-8893 200 x 50 µl reactions

For research purposes only.

Storage at –20°C Guaranteed for 12 months at –20°C in a constant temperature freezer (multiple freeze/thaw cycles not recommended)

Storage at +4°C Guaranteed for 4 months at 4°C (protected from light)

The iScript one-step RT-PCR kit with SYBR® Green is a convenient and highly sensitive solution for real-time quantitative PCR of RNA templates. cDNA synthesis and PCR amplification are carried out in the same tube. This kit is optimized to deliver maximum RT-PCR efficiency, sensitivity and specificity without compromising fluorescent signal. The proprietary reaction buffer has been specifically formulated to maximize activities of both the reverse transcriptase and the iTaq™ DNA polymerase, while minimizing the potential for primer-dimer and other nonspecific PCR artifacts.

2x SYBR® Green RT-PCR reaction mix includes the iTaq antibody-mediated hot-start DNA polymerase that sequesters activity prior to the initial PCR denaturation step. Upon heat activation, the antibody denatures irreversibly, releasing fully active and unmodified iTaq DNA polymerase. Highly specific amplification is essential for successful qRT-PCR with SYBR® Green I technology, since this dye binds to any double-stranded DNA generated during amplification.

Kit Contents

Reagents	**Description**
iScript reverse transcriptase for one-step RT-PCR (yellow cap)	Optimized 50x formulation of iScript MMLV for one-step RT-PCR procedures
2x SYBR® Green RT-PCR reaction mix (green cap)	2x reaction buffer containing 0.4 mM of each dNTP (dATP, dCTP, dGTP, dTTP), magnesium ions, iTaq DNA polymerase, 20 nM fluorescein, SYBR® Green I dye, stabilizers
Nuclease-free water	

Reaction Set Up

To maximize specificity, reactions should be assembled on ice.

Component	**Volume per Reaction**
2x SYBR® Green RT-PCR reaction mix	25 µl
Forward primer (300 nM final concentration)	Variable
Reverse primer (300 nM final concentration)	Variable
Nuclease-free water	Variable
RNA template (1 pg to 100 ng total RNA)	Variable
iScript reverse transcriptase for one-step RT-PCR	1 µl
Total Volume	**50 µl**

Reaction Protocol

Incubate complete reaction mix in a real-time thermal detection system as follows:

cDNA synthesis	10 min at 50°C
Reverse transcriptase inactivation	5 min at 95°C
PCR cycling and detection (30 to 45 cycles)	10 sec at 95°C 30 sec at 55°C-60°C (data collection step)
Melt curve analysis (optional)	1 min at 95°C 1 min at 55°C 10 sec at 55°C-95°C (80 cycles, increasing by 0.5°C each cycle)

Recommendations for Optimal Results Using the iScript One-Step RT-PCR Kit With SYBR® Green

Primers should be designed according to standard PCR guidelines with a length of 18-25 nucleotides, and a GC content of 40%-65%. Primer design should avoid internal secondary structure and complementarity at the 3' ends within each primer and primer pair. Optimal results may require titration of primer concentration between 100 and 500 nM. A final concentration of 300 nM per primer is effective for most reactions. In general, reaction efficiency and/or specificity can be optimized using equal concentrations of each primer. For best results, amplicon size should be limited to 50–200 bp.

Suggested input quantities of template are: 1 pg to 100 ng total RNA; 10 fg to 100 ng polyA(+) RNA.
First strand synthesis can be performed between 40°C and 52°C. Optimal results are generally obtained with a 10 min incubation at 50°C. Incubation at temperatures higher than 50°C can delay or eliminate the detection of some nonspecific amplification artifacts. However, this may also delay the C_t for detection of specific targets.

Thaw all components, except the iScript reverse transcriptase, at room temperature. Mix gently, but thoroughly, and then centrifuge at 4°C to collect contents to the bottom of the tube. Chill on ice before using.

Preparation of a reaction cocktail is crucial in quantitative PCR applications to reduce pipetting errors and maximize assay precision and accuracy. Assemble the reaction cocktail with all required components except sample template (total RNA) and dispense equal aliquots into each reaction tube. Add target sample to each reaction as the final step. Addition of sample as 5–10 µl volumes will improve assay precision. Replicate samples should be assembled as a master mix with a single addition of sample template.

To learn more about Bio-Rad's complete solution for amplification, visit our website:
www.bio-rad.com/amplification.

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