iScript™ One-Step RT-PCR Kit for Probes

50 x 50 µl reactions	170-8894
200 x 50 µl reactions	170-8895

For research purposes only.
Store at –20°C.

The iScript one-step RT-PCR kit for probes is a convenient and highly sensitive solution for real-time quantitative PCR of RNA templates using hybridization-based probe detection chemistries such as 5'-hydrolysis probes or molecular beacons. 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. The reaction buffer has been specifically formulated to maximize activities of both the iScript reverse transcriptase and the iTaq™ DNA polymerase, while minimizing the potential for primer-dimer and other non-specific PCR artifacts.

The 2x RT-PCR reaction mix for probes 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.

Storage and Stability

Store the iScript one-step RT-PCR kit for probes at –20°C in a constant temperature freezer. When stored under these conditions, the kit components are stable for a minimum of one year after ship date. For extended stability, the iScript reverse transcriptase for one-step RT-PCR may be stored at –70°C. For convenience, the 2x RT-PCR reaction mix for probes may be stored unfrozen at 2°C to 8°C for up to 6 months. Repeated freeze-thaw cycles should be avoided.

Kit Contents

Reagent	Description
iScript reverse transcriptase for one-step RT-PCR (yellow cap)	Optimized 50x formulation of iScript MMLV reverse transcriptase for one-step RT-PCR procedures
2x RT-PCR reaction mix for probes (blue cap)	2x reaction buffer containing 0.5 mM of each dNTP (dATP, dCTP, dGTP, dTTP), magnesium ions, iTaq DNA polymerase, stabilizers
Nuclease-free water	

Reaction Set Up

To avoid primer-dimers, reactions should be assembled on ice.

Component	Volume per reaction
2x RT-PCR reaction mix for probes	25 µl
Forward primer, 100 to 500 nM final concentration	variable
Reverse primer, 100 to 500 nM final concentration	variable
Probe, 50 to 200 nM final concentration	variable
Nuclease-free water	variable
RNA template	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
iScript reverse transcriptase inactivation:	5 min at 95°C
PCR cycling and detection (30 to 45 cycles):	10 to 15 sec at 95°C
	30 sec at 55°C to 60°C (data collection step)

Recommendations for optimal results using the iScript One-Step RT-PCR Kit for Probes

Probe and primers should be designed according to standard qPCR guidelines.

Suggested input quantities of template are: 1 pg to 1 µg 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-minute incubation at 50°C. Incubation at temperatures higher than 50°C can delay or eliminate the detection of some non-specific amplification artifacts. However, this may also delay the C_t for detection of specific targets. We also recommend a 5-minute incubation at 95°C to fully inactive the reverse transcriptase prior to PCR cycling.

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. Centrifuge again briefly at 4°C if needed.

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.

Reagents and Materials Not Supplied

Gene-specific primers and probe
Pipet tips, aerosol barrier tips, such as:
    Xcluda® Style B, 211-2006
Nuclease-free tubes or plates, such as:
    0.2 ml thin-wall tubes, 223-9473 or plates, 223-9441
RNA purification kit, such as:
    Aurum™ total RNA mini kit, 732-6820, or
    Aurum total RNA kit, 2 x 96 well, 732-6800

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

Bio-Rad Laboratories
2000 Alfred Nobel Drive, Hercules, CA 94547
510-741-1000