Product Profile

QIAGEN® OneStep *Ahead* RT-PCR Kit

The QIAGEN OneStep *Ahead* RT-PCR Kit provides a convenient format for highly sensitive and specific RT-PCR using any RNA. The kit contains optimized components that allow both reverse transcription and PCR amplification to take place in what is commonly referred to as a "one-step" reaction.

The QIAGEN OneStep *Ahead* RT-PCR Kit provides:

- Unique two-phase hot-start procedure for room-temperature setup
- Dedicated enzyme mix for increased specificity, sensitivity and fidelity
- Faster than ever 1-hour cycling protocol
- Visual pipetting control, resulting in fewer pipetting errors
- Duplex capability, enabling inclusion of internal control or reference gene

OneStep *Ahead* RT-PCR Procedure

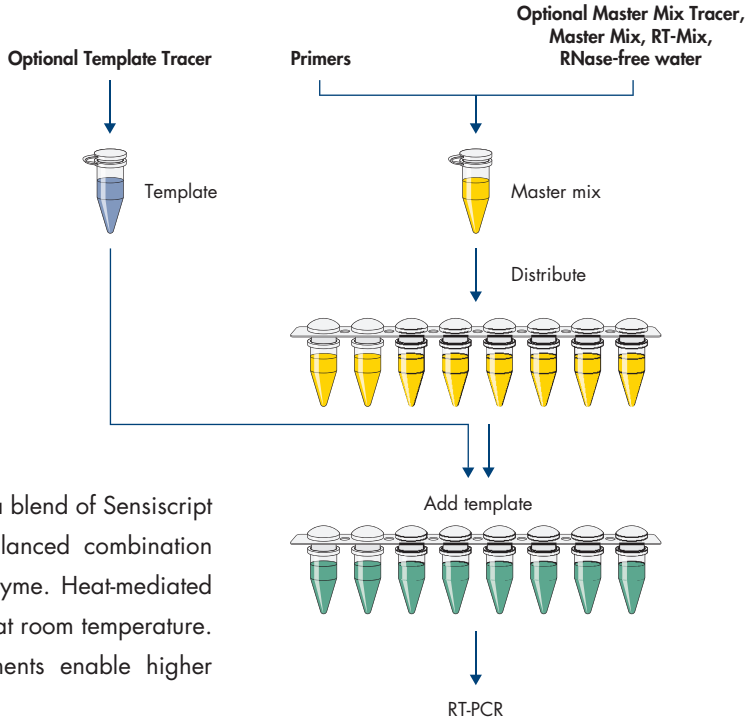

Figure 1. The OneStep *Ahead* RT-PCR Kit procedure.

The QIAGEN One-Step *Ahead* RT-PCR Kit contains a blend of Sensiscript and Omniscript Reverse Transcriptases, a well-balanced combination of *Taq* DNA Polymerases and a proofreading enzyme. Heat-mediated activation of these enzymes enables reaction setup at room temperature. The easy one-tube setup and optimized components enable higher sensitivity and successful results.

Convenience

The QIAGEN OneStep *Ahead* RT-PCR Kit allows fast and easy RT-PCR setup whatever the application – virus detection, molecular diagnostics research or gene expression analysis – just add the RT-enzyme mix, primers and template to the Master Mix in one tube and start the ▷

thermal-cycler program. Reaction setup can conveniently be done at room- temperature and the optional master mix and template tracers minimize pipetting errors. The reaction mixture contains all of the reagents required for both reverse transcription and PCR (see Figure 1 for a schematic description of the procedure).

Performance

The QIAGEN OneStep *Ahead* RT-PCR Kit provides a convenient format for highly sensitive and specific RT-PCR using any RNA template. The kit includes optimized components that allow both reverse transcription and PCR amplification to take place in the same reaction mix in a "one-step" reaction. A unique enzyme combination and specially developed reaction buffer ensure efficient, highly specific reverse transcription and PCR in one tube, without the need for optimization (see Figures 2 and 3).

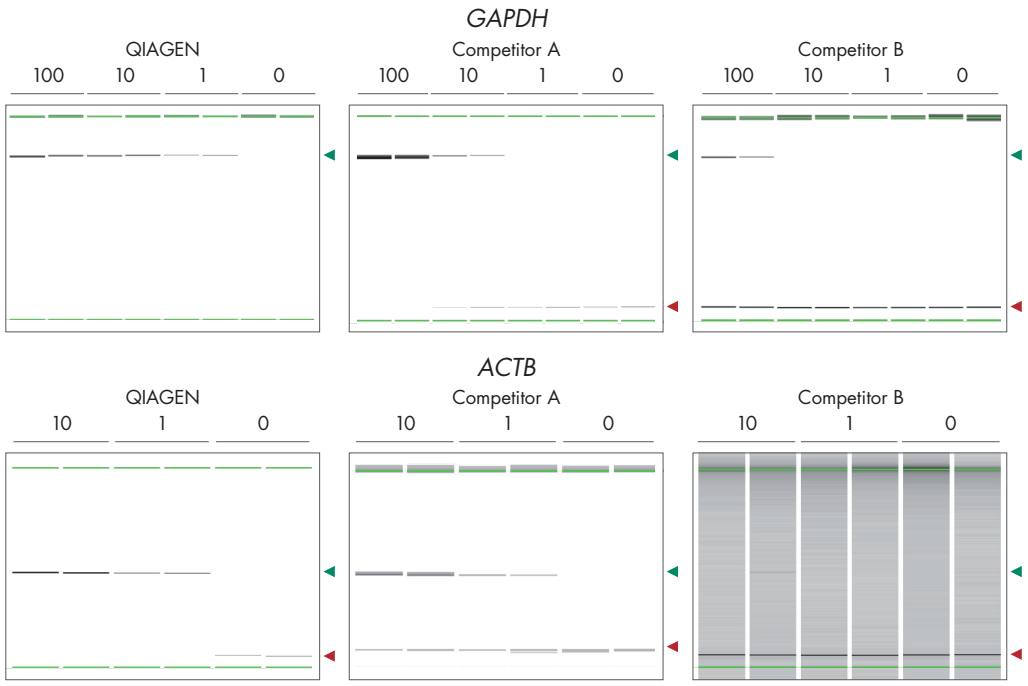

Figure 2. Superior sensitivity. Indicated amounts of HeLa total RNA (in pg) were used as template for amplification of GAPDH (831 bp) and ACTB (295 bp) in duplicate, according to the suppliers' instructions. Green arrows indicate specific product, red arrows indicate primer-dimers. Analysis was performed using the QIAxcel.

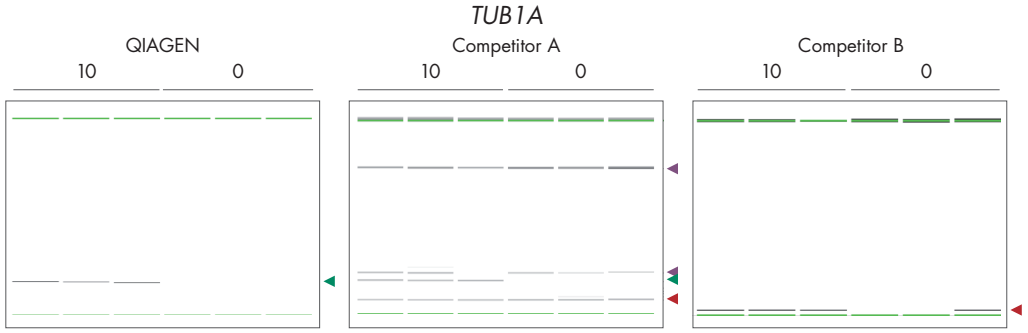

Figure 3. Superior specificity. Indicated amounts of HeLa total RNA (in ng) were used as template for amplification of EIF2B4 (107 bp) in triplicates, according to the suppliers' instructions. Green arrows indicate specific product, red arrows indicate primer-dimers, purple arrows indicate nonspecific amplification products. Analysis was performed using the QIAxcel.

For the reverse-transcription step, both Omniscript and Sensiscript are functionalized with an RT-blocker, which keeps them in an inactive state and prevents nonspecific transcription at ambient temperature. When the reaction is heated to 50°C, the RT-blocker dissociates from the RT enzymes, rendering them fully active.

The PCR amplification step is catalyzed by a well-balanced blend of three different DNA polymerases. QuantiNova DNA Polymerase is provided in an antibody-mediated, inactive state and has no enzymatic activity at ambient temperature. Activation of this enzyme occurs after a 5-minute incubation step at 95°C, allowing highly specific amplification from the first cycle. HotStarTaq DNA Polymerase activation occurs gradually and ensures high yields of the PCR product. The high-fidelity proofreading DNA Polymerase, with 3'→5' exonuclease activity, is also heat-activated by the initial 5-minute incubation step at 95°C, ensuring superior amplification accuracy and processivity. The innovative, dual-cation PCR buffer provided in the master mix ensures high yields of specific PCR products over a wide range of annealing temperatures. Suboptimal RT-PCR is improved using Q-Solution, a unique additive that facilitates reverse transcription and amplification of templates with a high GC content or a high degree of secondary structure.

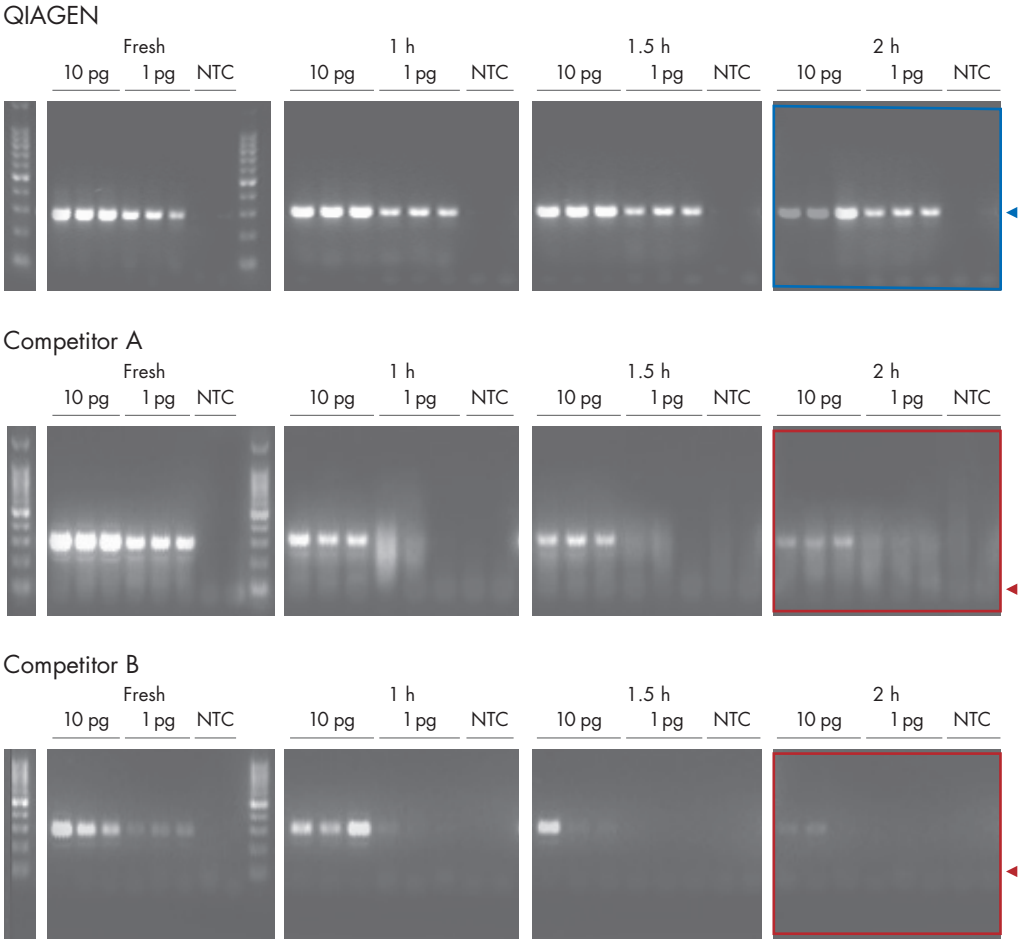

Figure 4. Reaction setup at room temperature. Samples can be conveniently set up and kept at room temperature before cycling, enabling use of the kit in automated robotic workflows. HeLa total RNA (10 and 1 pg) was used as template for amplification of ACTB in triplicates, according to the suppliers' instructions. Reactions were either setup on ice ("Fresh") or left at room temperature for the times indicated before analysis on a 2% agarose gel.

Reaction setup at room temperature adds convenience and facilitates use of the kit in automated workflows. The master mix includes buffer, dNTPs and DNA polymerases. The RT-Mix, which includes the reverse transcription enzymes as well as the RNase-inhibitor, is provided separately to allow for minus RT controls. To set up the reaction, simply add the primers, target RNA and RT-Mix to the master mix.

Integrated control features prevent artifacts and ensure reliable results

The optional tracking system, comprising a Master Mix Tracer and Template Tracer for visual identification of correct pipetting, minimizes errors during setup (see Figure 5). The chemistry is optimized for duplex PCR to enable co-amplification of an internal positive control with every reaction. The included RNase inhibitor prevents RNA decay caused by accidental RNase contamination.

Figure 5. Optional pipetting control. The kit comes with optional pipetting controls. The Master Mix Tracer is an inert orange dye that can be added to the Master Mix. The Template Tracer is an inert blue dye that can be added to the template. When adding the template to the Master Mix, the color turns green, providing a visual indication of correct pipetting. In addition, both dyes allow tracking during gel electrophoresis. The dyes run at approximately 50 bp (orange) and 4000 bp (blue) on a 1% agarose gel.

Ordering Information

Product	Contents	Cat. no.
QIAGEN OneStep *Ahead* RT-PCR Kit (50)	6 vials for 50 reactions: 1 x 500 µl OneStep *Ahead* RT-PCR Master Mix, 1 x 50 µl OneStep *Ahead* RT Mix, 1 x 200 µl Template Tracer, 1 x 50 µl Master Mix Tracer, 1 x 1.9 ml water, 1 x 400 µl Q-Solution	220211
QIAGEN OneStep *Ahead* RT-PCR Kit (200)	8 vials for 200 reactions: 2 x 1 ml OneStep *Ahead* RT-PCR Master Mix, 1 x 200 µl OneStep *Ahead* RT Mix, 1 x 200 µl Template Tracer, 1 x 50 µl Master Mix Tracer, 2 x 1.9 ml water, 1 x 2 ml Q-Solution	220213
QIAGEN OneStep *Ahead* RT-PCR Kit (2000)	*75* vials for 2000 x 25 µl reactions: 20 x 1 ml OneStep *Ahead* RT-PCR Master Mix, 10 x 200 µl OneStep *Ahead* RT Mix, 10 x 200 µl Template Tracer, 10 x 50 µl Master Mix Tracer, 20 x 1.9 ml water, 5 x 2 ml Q-Solution	220216

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.