

Evaluation of the AmpliStar One-Step qRT-PCR Kit for viral RNA diagnostics

Introduction

One-Step quantitative RT-PCR (qRT-PCR) is a powerful technique for detection of one or a small number of targets in a large number of samples. Because the entire reaction takes place in a single, closed well there is less experimental variation and a lower risk of cross-contamination. This makes One-Step qRT-PCR an excellent choice for high-throughput diagnostic assays.

In order to make reliable calls, a critical requirement for diagnostic assays is that the reagents used offer the highest possible robustness and consistency of the obtained data. This application note shows the results of an evaluation by a user of the AmpliStar One-Step qRT-PCR Kit for detection of RNA virus.

Results

Consistency between wells, lots and vials

The consistency of the AmpliStar One-Step qRT-PCR Kit was evaluated on a sample containing RNA virus and spiked with an internal control. For two different kit production lots (A and B), and two different vials of lot B eight replicates each were assayed. The internal control was detected in a second channel. A no-template control (NTC) was also included. Figure 1 shows that all 24 data points were in very close agreement, both for the RNA virus target and the internal control. Only in the plateau phase the curves diverged slightly, mostly due to variability between wells.

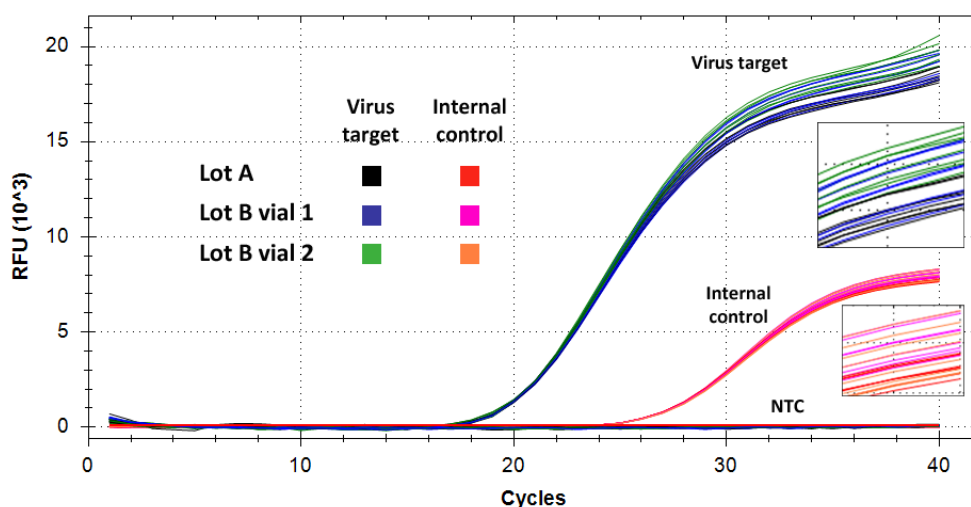


Figure 1

qRT-PCR data consistency across 8 replicates of different lots and vials.

Inset shows enlarged plateau phase

Robustness towards temperature variability

In the same assay, the AmpliStar One-Step qRT-PCR Kit (Figure 2, in green) showed a substantially higher tolerance towards variability in annealing temperature (T_a) than a similar kit from a leading competitor (in blue). In the experiment, T_a was varied between 56.4 and 62.9°C. Except for the highest T_a , all AmpliStar curves were very closely aligned. This indicates that measurements are much less sensitive to cycle edge effects or other temperature deviations, which further contributes to the reliability of the results.

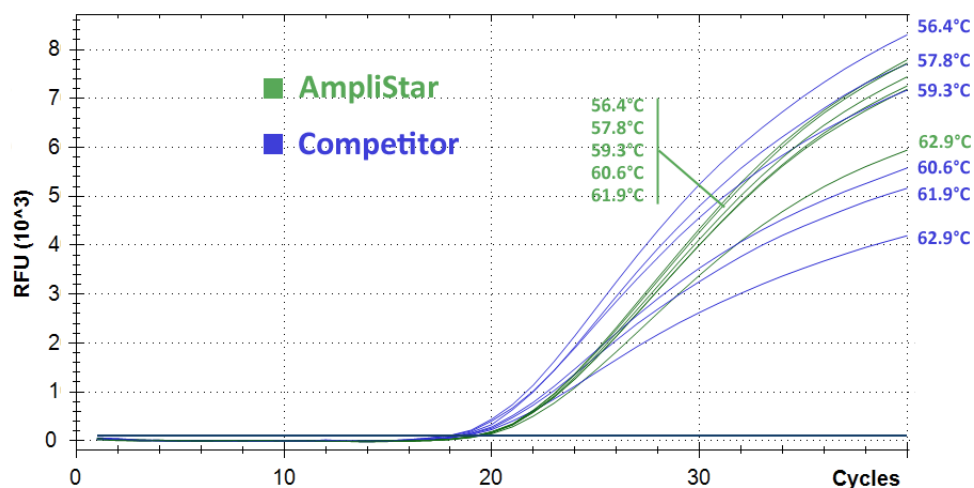


Figure 2

AmpliStar One-Step qRT-PCR assay data are barely influenced by variability in annealing temperature.

Kit components

- Enzyme mix
 - RNase H- Reverse Transcriptase
 - Hot-Start Taq DNA Polymerase
 - RNase inhibitor
- Reaction buffer with dNTPs

Key benefits

- Excellent reproducibility
- High sensitivity
- 7-log dynamic range
- Multiplex up to 5 targets

Ordering information

Cat. no.	Description	Size
WB 14150	AmpliStar One-Step qRT-PCR Kit	500 reactions