Alien Reference RNA QRT-PCR Detection Kit for Monitoring the Overall Performance of QRT-PCR Assays

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Introduction

Exogenous RNA controls are increasingly used in QRT-PCR experiments to normalize variations that arise due to the presence of spurious inhibitors. In addition, RNA controls are added to all QRT-PCR experiments to standardize data. The International Oligonucleotide & Analytical Applications Database (IOLAND) has identified the use of exogenous standards as a key strategy to ensure the accuracy of QRT-PCR data. Therefore, it is recommended to use exogenous RNA controls for each new QRT-PCR experiment. When inhibitors are present, the Alien QRT-PCR Inhibitor Alert Kit detects a delay in the threshold cycle (Ct) values between Alien RNA amplification and the presence of simple RNA exudation. The Alien Probe insert is an efficient RNA-RNA exudation chemistry, which provides high sensitivity but does not allow multiple amplification or Alien RNA real-time detection.

We recently expanded our line of exogenous controls to include Alien Reference RNA, pre-bound Alien primers (and Alien RNA labeled for detection of endogenous Alien RNA transcript in 1-step and 2-step QRT-PCR assays. As we will demonstrate, the Alien probe system generalizes standard curves showing linear dynamic range (from 0 to 10,000,000) and single-step detection of Alien RNA in 2-step QRT-PCR. In addition, the Alien probe system is sensitive to a variety of QRT-PCR inhibitors, and allows multiplex detection of Alien and sample RNAs in the same tube.

Results

Alien Reference RNA is a 500 bp in vitro transcribed RNA from a computer generated sequence that possesses 50% GC-content and is non-homologous to sequences in GenBank.

Part 1: Alien Reference RNA does not cross hybridize to human, mouse, or rat sequences

Alien Reference RNA is a 500 bp in vitro transcribed from a computer generated sequence that possesses 50% GC-content and is non-homologous to sequences in GenBank.

Part 2: Alien Reference RNA is devoid of contaminating DNA

Amplification reactions contained 100 ng of Alien RNA primers synthesized 105 copies of Alien RNA, or 50 ng of Human, Mouse, or Rat Universal Reference RNA (Stratagene) in separate tubes. Amplifications were performed with the Brilliant II SYBR Green QRT-PCR Master Mix (Livak). No amplification products were detected using Human, Mouse, or Rat Universal Reference RNA.

Part 3: Alien Reference RNA primer/probe sets exhibit high amplification efficiency, sensitivity, and dynamic range

a) 1-step and 2-step singleplex QRT-PCR

A) FAM probe

In panel A, a Brilliant II SYBR Green QRT-PCR Master Mix (1-step) was used to generate a standard curve with the Alien Reference RNA FAM primer/probe set from 109 to 10 copies (7 orders of magnitude). In panel B, Brilliant II QRT-PCR Master Mix (2-step) was used to generate a standard curve with the Alien Reference RNA FAM primer/probe set from 109 to 10 copies (7 orders of magnitude). The 2-step QRT-PCR resulted in amplification efficiency of 95%, R² of 0.998, and detection limit of 10 copies. The 2-step QRT-PCR generated amplification efficiency of 100% R² of 0.998, and detection limit of 1 step.

b) 2-step multiplex QRT-PCR

Amplification reactions were performed using Brilliant II SYBR Green QRT-PCR Master Mix (1-step). 105 copies of Alien Reference RNA and 10ng Human Reference RNA were added to the cDNA synthesis reactions. EDTA was titrated into the reactions at the concentrations indicated above. Inhibition of amplification of both Alien Reference RNA and cyclophilin was observed at 10mM EDTA similar to the results obtained above (part 4, section c).

Results-continued

c) EDTA in one-step singleplex QRT-PCR

Amplification reactions were performed using Brilliant II SYBR Green QRT-PCR Master Mix (1-step). 105 copies of Alien Reference RNA and 10ng Human Reference RNA were added to the cDNA synthesis reactions. EDTA was titrated into the reactions at the concentrations indicated above. Inhibition of amplification of both Alien Reference RNA and cyclophilin was observed at 10mM EDTA similar to the results obtained above (part 4, section c).

Results-continued

d) EDRA in two-step multiplex QRT-PCR

Amplification reactions were performed using Brilliant II SYBR Green QRT-PCR Master Mix (1-step). 105 copies of Alien Reference RNA and 10ng Human Reference RNA were added to the cDNA synthesis reactions. EDTA was titrated into the reactions at the concentrations indicated above. Inhibition of amplification of both Alien Reference RNA and cyclophilin was observed at 10mM EDTA similar to the results obtained above (part 4, section c).

Conclusions

- Alien Reference RNA QRT-PCR detection kit is used for validating the quality of experimental RNA samples, improving the quality of QRT-PCR data, and monitoring the overall performance of QRT-PCR assay reagents and instrumentation.
- Alien Reference RNA is produced in large lots and subject to stringent quality control measures to ensure the availability of consistent Alien RNA relative to near term experimental studies.
- The kit includes an exponential RNA standard, two primers and a TaqMan probe. The TaqMan probe is provided with FAM or VIC or ROX dye for multiplexing flexibility.
- When multiplexing Alien Reference RNA with a gene of interest (GOI), general multiplexing guidelines should be followed to ensure compatibility. These guidelines include using spectrally distinct fluorophores, a multiplex QPCR kit (e.g., Brilliant Multiplex kit), and limiting primer/probe concentration for higher abundance targets.
- If the presence of an inhibitor in RNA samples is suspected, researchers are encouraged to re-purify or dilute the RNA sample (if target abundance allows it) and repeat the amplification. Furthermore, a two-step QRT-PCR format may be more appropriate to allow identification of QRT-PCR inhibitors.
- Alien Reference RNA QRT-PCR detection kit will be commercially available on 11/26/08.

References


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