Alien® QRT-PCR Inhibitor Alert

Detect Inhibitors in RNA Samples for QRT-PCR

+ Universal external RNA control
+ Sensitive to common inhibitors
+ Known copy number provided
+ Ideal reference tool for assay standardization

OUR ALIEN® QRT-PCR INHIBITOR ALERT IS A HIGH-QUALITY EXTERNAL CONTROL FOR DETECTING INHIBITORS IN RNA SAMPLES FOR QRT-PCR EXPERIMENTS.

Real-time quantitative reverse transcription PCR (QRT-PCR) is an established method for mRNA quantitation in biological samples. The quality of your RNA template is the most important determinant of the reproducibility and biological relevance of your subsequent QRT-PCR results. Our Alien® QRT-PCR Inhibitor Alert is a useful tool as an external RNA control to detect inhibition.

Nucleic acid templates extracted from a variety of biological samples have been shown to contain inhibitors that may inhibit either the reverse transcription or PCR, or both steps. These DNA polymerase inhibitors, along with the variations in nucleic acid extraction efficiency, can lead to misinterpretation of the expression of levels of target sequences. Historically, "housekeeping genes" have been used widely as internal RNA references. However, their expression levels are found to vary significantly across tissues and different development stages, as well as between individuals. If you plan to use housekeeping genes, they must be validated for the specific experimental set, and it will probably be necessary for you to choose more than one to use in your experiment.

As an alternative to an endogenous RNA reference, we have developed an exogenous reference mRNA for QRT-PCR analysis. Our Alien RNA transcript is an *in vitro* transcribed RNA that is non-homologous to the sequences currently available in GenBank. A known amount of Alien RNA is amplified in the presence of an RNA sample of interest. An increase in the threshold cycle (Ct value) for amplification of the Alien RNA in the sample compared with the Alien RNA alone will be an indicator of the presence of inhibitory substances in the sample. The Alien QRT-PCR Inhibitor Alert can be used to detect inhibitors in both one-step (single-tube) and two-step (two-tube) QRT-PCR assays that employ SYBR® Green dye for detection.

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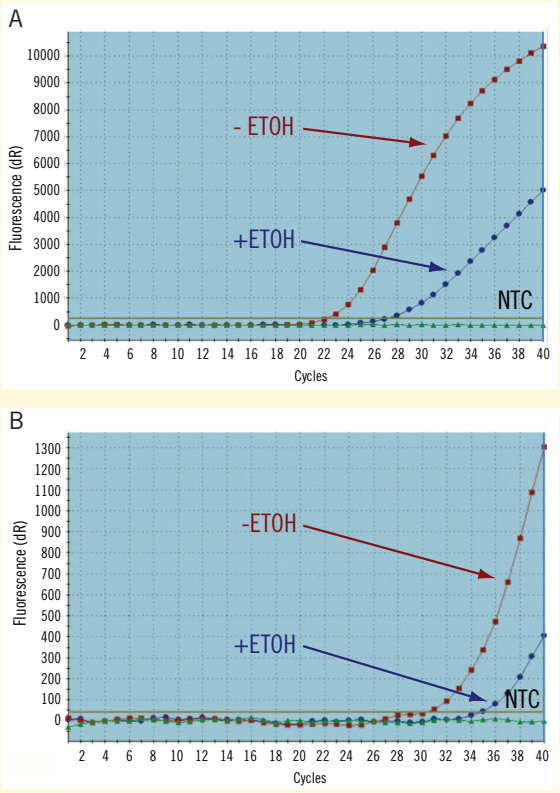

Figure 1
Amplifications of Alien RNA and TBP are Inhibited in the Presence of Ethanol
Amplifications were performed in the presence or absence of 2.5% ethanol. Reactions contained 10^5 copies of Alien RNA, 50 ng Stratagene® QPCR Reference Human Total RNA, 100 nM Alien primer mix (A) or TBP primers (B) using Brilliant® SYBR® Green QRT-PCR 1-step Master Mix.

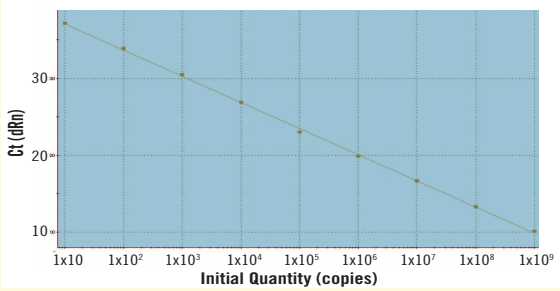

Figure 2
Standard Curve Using Alien® RNA and Brilliant® SYBR® Green QRT-PCR 1-Step Master Mix
A serial dilution of the Alien® RNA ranging from 1 to10^9 copies was used. The standard curve spans eight orders of magnitude with an Rsq of 0.997 and amplification efficiency of 98.7%.

Inhibitor Alert

There are a variety of inhibitors that can affect the efficiency of your QRT-PCR reactions and may be co-purified with RNA samples, depending on the source of starting material, the methods of extraction, and variable factors. Common QRT-PCR inhibitors include phenol, ethanol, guanidine, heparin, and EDTA.

To demonstrate the effectiveness of the Alien QRT-PCR Inhibitor Alert as an external control, we added 2.5% ethanol to a human total RNA sample and monitored the amplification of the Alien RNA and TBP (TATA box Binding Protein) in the presence and absence of the inhibitor. In the presence of 2.5% ethanol, the amplification of Alien RNA was inhibited by as much as 6 Ct values (Figure 1A). When the TBP target was amplified, a delay of 3 Ct values was observed in the presence of 2.5% ethanol (Figure 1B). Because the amplification of Alien RNA is highly sensitive to a number of common QRT-PCR inhibitors, it is a valuable tool in determining the quality of different RNA samples when you are studying gene expression levels with samples obtained from various sources.

When there are inhibitors in your RNA samples, you can either further purify the samples to remove the inhibitors or dilute the samples. Dilution is recommended only when your gene of interest is present in high abundance in your samples.

Assay Standardization

The Alien QRT-PCR Inhibitor Alert is ideally suited for your assay standardization applications. Using the Alien QRT-PCR Inhibitor Alert as a reference control to generate standard curves allows you to conduct data comparisons between multiple experiments, across platforms, and between laboratories. The Alien RNA is produced in large lots and subject to stringent quality-control measures to ensure the availability of consistent reference RNA material over long-term experimental studies. By using our Alien RNA control transcript and Alien primer mix, you can generate a standard curve that provides 8 orders of magnitude in dynamic range (Figure 2). Since Alien RNA has no significant homology to known sequences, you can use it as your standardization for real-time QRT-PCR experiments.

Choose Alien® QRT-PCR Inhibitor Alert with our Brilliant® SYBR® Green 1-Step or 2-Step QRT-PCR Master Mixes for best results.

Alien® QRT-PCR Inhibitor Alert Products	Catalog No.
Alien® QRT-PCR Inhibitor Alert	300600
Brilliant® SYBR® Green QRT-PCR Master Mix, 1-step, with Alien Control	300601
Brilliant® SYBR® Green QRT-PCR Master Mix, 2-step, with Alien Control	300602

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Please note that the Mx3000P® QPCR System and the Mx3005P® QPCR System are not offered, sold, and imported in/to Germany.

For a list of worldwide distributors, please visit our website.

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