

Ask Frank

by Fred and Hank



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Questions about EPICENTRE Biotechnologies' new MessageBOOSTER™ cDNA Synthesis Kit for qPCR

Q. Why is the MessageBOOSTER™ cDNA Synthesis Kit such a benefit for quantitative real-time PCR (qPCR)?

A. For a number of reasons: With even low-abundance transcripts the MessageBOOSTER Kit can produce enough cDNA for sensitive and reproducible qPCR starting with total RNA from 1 to 50 cells (10 – 500 pg). It is important to note that the MessageBOOSTER amplification process is linear, which means that the overall gene expression profile is preserved. Each reaction starting with total RNA from one cell, will produce enough cDNA for up to 10 qPCR experiments.

Q. How does the MessageBOOSTER cDNA Synthesis Kit work?

A. Initially, poly(A) RNA in the total RNA sample undergoes a modified and improved "Eberwine-type" T7-based RNA amplification procedure to produce antisense RNA (aRNA, also called complementary RNA [cRNA]). The synthesized aRNA is then spin column purified and used in a cDNA synthesis reaction using EPICENTRE's MMLV (Moloney Murine Leukemia Virus) Reverse Transcriptase Plus (MMLV-RT Plus) with random primers. This creates a population of cDNA molecules that are an excellent, high-fidelity representation of the gene expression profile of the cell. The cDNA generated can then be used directly in qPCR applications with SYBR® Green I dye or fluorescent probe detection.

Q. Once I have synthesized the cDNA with the MessageBOOSTER Kit, how much of it should I use in my qPCR?

A. The cDNA produced by a MessageBOOSTER reaction is in a final volume of ~ 5.5 µl. The amount of cDNA to add to a qPCR reaction is dependent on the amount of starting total RNA in the MessageBOOSTER reaction and on the abundance of the transcript of interest and should be determined empirically. For example, when starting with 10 pg of total RNA (about 1 cell equivalent), to detect low- or medium-abundance transcripts you would use 1 µl of the MessageBOOSTER final reaction volume. For the detection of high-abundance transcripts, you would dilute the MessageBOOSTER final reaction volume 1:10, and then use 1 µl in your qPCR assays. For more details see the article in EPICENTRE Forum 13.1.¹

Q. In order to obtain optimal results with the MessageBOOSTER cDNA Synthesis Kit, what are some of the important factors I should consider?

A. Before using up precious sample, familiarize yourself with the kit by running a control reaction. Use no more than 500 pg of total RNA (~50 cells) per reaction, and remember that the success of an RNA amplification reaction is strongly influenced by the quality of the input RNA.

Q. For best results, how should I design my primers for qPCR when using input cDNA synthesized by a MessageBOOSTER reaction?

A. The amplified RNA used to make cDNA in a MessageBOOSTER Kit reaction will have a 3-prime bias. For optimal qPCR sensitivity, we recommend selecting primers that prime within 500 bases of the 3'-end of the mRNA.

Primers for sequences >500 bases from the 3'-end of the mRNA(s) may give reduced sensitivity.

Q. You are using a wild-type MMLV-RT in the second cDNA reaction, and then using RNase H to degrade the RNA portion of the reaction afterwards. Why is this? Doesn't wild-type MMLV have RNase H activity?

A. While EPICENTRE's MMLV-RT Plus does have RNase H activity, using additional RNase H after the reverse transcription reaction has been demonstrated to enhance the removal of the RNA from the RNA:DNA hybrid and yield more sensitive qPCR results.

Q. Does the cDNA produced in a MessageBOOSTER reaction require additional cleanup after the RNase H treatment before use in qPCR?

A. No. The cDNA may be used directly as template in your qPCR reactions.

Reference

1. Grunenwald, H. *et al.*, (2006) EPICENTRE Forum 13(1), 7.

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FOR MORE INFORMATION ON
THE MESSAGEBOOSTER™ cDNA
SYNTHESIS KIT FOR qPCR

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