Ask Frank

by Fred and Hank

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Questions about TargetAmp™ Aminoallyl-aRNA Amplification Kits

We strive to provide our customers with the most accurate technical support – both in selecting an appropriate product and answering your technical questions. If you need more information about the TargetAmp products, or any EPICENTRE products, please contact us.
Fred and Hank

Q. When should I use the 1-Round vs. the 2-Round TargetAmp™ aRNA Amplification Kit?

A. Use the TargetAmp 1-Round aRNA Amplification Kit to produce micrograms of antisense RNA (aRNA) from as few as 1000 cells or 25 ng total cellular RNA. The TargetAmp 2-Round Kit efficiently amplifies RNA from one cell, or as little as 10 to 500 pg of total cellular RNA. For more specific yield data, please see the Tables on pages 4 and 6. If you are planning to compare multiple samples, be sure that all of the samples are prepared by the same procedure. (i.e., all 1-Round or all 2-Round)

Q. What is the best way to preserve samples and purify RNA for use with the new TargetAmp™ aRNA Amplification Kits?

A. Any RNA Purification Kit that produces highly purified, full-length RNA will work fine. We recommend our ArrayPure™ RNA Purification Kit, which can purify RNA from as little as one cell (see page 8), or our MasterPure™ RNA Purification Kit for >10,000 cells. When using any salt-based extraction system, like the ArrayPure and MasterPure Kits, we advise not using RNA*later*®-Ice to preserve the samples prior to extraction, because it may interfere with the proteinase K digestion step. Instead, use a snap-freeze technique with a dry ice/ethanol bath followed by storage at -80°C until RNA Purification. The purified RNA should be resuspended in water, and be free of salts, ethanol, and genomic DNA.

Q. In the TargetAmp procedure, the second-strand cDNA synthesis reaction (first round) is incubated at 65°C. Why is that? Other procedures incubate this reaction at 16°C.

A. The TargetAmp Kits use a unique, proprietary thermostable DNA polymerase that works optimally at 65°C. This enzyme contributes significantly to the very high aRNA yields obtained with the TargetAmp Kits.

Q. What amount of RNA amplification can I expect with the TargetAmp 1-Round and 2-Round aRNA Amplification Kits?

A. With the 1-Round Amplification Kit, you can expect at least 5000-fold amplification of the poly(A) RNA; with the 2-Round Kit, at least $5x10^6$-fold amplification of the poly(A) RNA is typical.

Q. How can I calculate the fold-amplification of aRNA that I get from my starting total RNA?

A. Depending upon the tissue or cell type being used, the poly(A) RNA fraction can be from 1% to 5% of the total RNA in a cell. Thus, when calculating the fold-amplification of a TargetAmp aRNA amplification, consider the amount of the poly(A) RNA, not the total RNA. From 100 pg of total RNA, let's estimate that the starting poly(A) RNA is approximately 3 pg (3%). If amplification produces 20 µg of RNA, the procedure resulted in ~7 $x10^6$-fold amplification of the poly(A) RNA.

Q. Why is the non-specific background amplification so low with the TargetAmp 2-Round aRNA Amplification Kit?

A. We use a proprietary background reduction chemistry to greatly reduce, or even eliminate, this problem.

Q. Can I use the TargetAmp Kits to make biotin-aRNA for use with Affymetrix GeneChip® arrays?

A. Yes, two TargetAmp Kits produce aminoallyl-aRNA, which can be readily conjugated to Biotin-X-X-NHS to produce biotin-aRNA (See page 7). The biotin-aRNA can be used as target on GeneChip® arrays.

Q. Can I quantitate my aminoallyl-labeled RNA by real-time RT-PCR?

A. Yes, see the article on page 20 for more information on this technique.

Find out more about the TargetAmp™ 1-Round aRNA Amplification Kits on page 6 and the TargetAmp™ 2-Round Kits on page 4.

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