

Ask Frank

by Fred and Hank



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Questions about Labeling anti-sense RNA (aRNA; also called cRNA) using EPICENTRE Biotechnologies' TargetAmp™ aRNA Amplification Kits

Q. Is it possible to incorporate biotin-UTP directly into the aRNA produced by the TargetAmp™ kits?

A. Yes. Both the TargetAmp™ 1-Round & 2-Round™ aRNA Amplification Kits can be modified for direct incorporation of biotin-UTP (provided by the user). Additionally, EPICENTRE's new TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 provides a UTP/biotin-UTP PreMix for optimized labeling and signal intensity. It will produce microgram amounts of biotin-aRNA from as little as 25 ng of total RNA for use on Affymetrix® GeneChip® arrays, Illumina® BeadChips, and other microarray platforms.

Q. Can you double-label aRNA with biotin-UTP and biotin-CTP?

A. Yes. Data in the literature indicate that the contribution of biotin-CTP to the overall signal intensity is much lower than that of biotin-UTP; however, incorporation of biotin-CTP may have some benefits for the detection of low-abundance targets. In addition to using biotin-UTP, 25-30% of the CTP can be substituted with biotin-16-CTP without decrease of yield in the 1-round amplification reactions.

Q. What is the recommended ratio of UTP to biotin-UTP?

A. The best ratio of UTP to biotin-UTP can vary a bit based on the nucleotide concentration and the GC content of the transcription templates. To achieve good overall signal intensity without compromising aRNA yield and length, between 25% to 40% of the UTP can be replaced with biotin-16-UTP (corresponding to

ratios of UTP/biotin-16-UTP of between 3:1 and 1.5:1) in the 1-round TargetAmp protocol.

Q. What are the advantages of aminoallyl-labeling over direct incorporation of a labeled-NTP?

A. The aminoallyl method for indirect labeling of the target nucleic acid has become increasingly popular because it has important advantages over direct incorporation of a biotin- or dye-labeled NTP. Aminoallyl-UTP is more efficiently incorporated into the aRNA during the *in vitro* transcription reaction than labeled nucleotides. Additionally, conjugation of an amine-reactive N-hydroxysuccinimide (NHS) ester of biotin (e.g., Biotin-X-X-NHS; EPICENTRE), Cy-NHS or other fluorescent dye-NHS to aminoallyl-aRNA (AA-aRNA) is a much less expensive way to label the target compared to direct incorporation of labeled nucleotides.

Q. What are the advantages of direct labeling of aRNA?

A. Labeling by direct incorporation is faster. Conjugation of aminoallyl-aRNA to aminoreactive biotin- or dye-derivatives after *in vitro* transcription requires a 1 hour incubation, and an additional clean-up step compared to direct incorporation. Furthermore, labeling aRNA by direct incorporation does not require the use of toxic reagents (such as dimethyl sulfoxide), which are used in the indirect labeling protocol.

Q. Can I use the TargetAmp Kits for producing Cy-labeled aRNA?

A. Yes. The TargetAmp™ 1-Round & the TargetAmp™ 2-Round Aminoallyl-aRNA

Amplification Kits produce AA-aRNA, which can be readily labeled with Cy-NHS or other types of fluorescent dye-NHS (provided by the user).

Q. What is the best method to clean up the labeled aRNA?

A. aRNA labeled by direct biotin-UTP incorporation is usually cleaned up on silica spin-columns. After coupling AA-aRNA to biotin- or dye-NHS, the labeled RNA can be cleaned-up using a spin-column, or with standard ion-exchange or gel filtration methods. Another common approach is to use microconcentrators.

Q. Are there any specific precautions one must take to ensure the efficiency of the aminoallyl/N-hydroxysuccinimide coupling reaction?

A. Biotin-X-X-NHS is readily hydrolyzed by water and can react with nucleophilic compounds, for example the amino groups of Tris buffers. Biotin-X-X-NHS should be dissolved in dry dimethyl sulfoxide (DMSO) as close to the time of use as possible. Once dissolved in DMSO or other solvent, its stability is entirely dependent on the continued absence of water or other nucleophilic compounds. Since DMSO is extremely hygroscopic and quickly takes up water vapor from the air, we recommend using Biotin-X-X-NHS that has been freshly dissolved in dry DMSO. If the Biotin-X-X-NHS has been dissolved and stored in DMSO, the stability of the Biotin-X-X-NHS should be validated prior to using it for biotinylation of aminoallyl-aRNA from a rare or precious sample.