TC RNA Phosphoramidite User Guide

RNA Synthesis

The synthesis cycle for TC RNA oligonucleotides consists of the same series of reactions as the cycle that is employed for DNA monomers. However, the rate of coupling for TC-RNA monomers is slower, compared to that of DNA monomers, so a coupling time of 3 minutes for TC RNA monomers is recommended compared to 90 seconds for DNA monomers. With the exception of the TC RNA monomers, supports (compatible with TC-chemistry, 2'-O-Acetyl protected RNA support or standard DNA support) and Diluent, RNA synthesis is accomplished with the same reagents as DNA synthesis.

All TC RNA phosphoramidites from SAFC® Proligo® Reagent are diluted with a mixture of dry acetonitrile and toluene (1:1 v/v), TC Amidite Diluent.

Method

1. Use anhydrous TC Amidite Diluent (L080000-100ml) or pure toluene (water content ≤ 30ppm) as diluents. It is important to maintain anhydrous conditions while dissolving TC RNA amidites.

2. SAFC recommends TC Amidite Diluent for solution up to 0.1M concentration.

   a. TC A *9.5mL/g*
   b. TC C *10.4mL/g*
   c. TC G *9.7mL/g*
   d. TC U *10.8mL/g*

   II 0.2M concentration 100% toluene is recommended.

3. Gently swirl the vial until the powder is completely dissolved.

4. Attach the dissolved phosphoramidite to the appropriate position on the synthesizer. Ensure that the delivery line to the synthesis chamber is sufficiently primed.

5. Once TC RNA phosphoramidite has been dissolved and placed on your instrument, the phosphoramidite should be used within 3 days.

6. Enter the sequence of the RNA oligonucleotide you wish to synthesize. A minimum coupling time of 3 minutes is recommended for 2'-O-TC-protected RNA amidites.

7. Proceed as you would with a standard DNA oligonucleotide synthesis. Depending on your intended further use of the oligomer, you can choose either DMT-On or DMT-Off procedures. The coupling efficiency of RNA monomers may be determined by standard dimethoxytrityl cation assays.

8. Cleave from the support and deprotect the RNA oligonucleotide with a neat Ethylenediamine (EDA; Aldrich 391085) at room temperature for 2 hours. It is not recommended to run cleavage and deprotection over night. **It is essential not to push the EDA through the column. Please only wet the support and leave the column undisturbed for 2 hours**
9. **It is essential to employ sterile conditions from this step forward.** After the cleavage and deprotection is completed, flush the remaining EDA from the column and wash the support with dry acetonitrile.

10. Dry the support/column by flushing argon or nitrogen for about 10 min.

11. Elute crude oligo from the column with buffer to receive optimal results or with sterile water