

Preparing RNA for Use

The 2'-protecting groups are completely removed using aqueous buffers in less than 30 minutes. (For discussion of the mechanism, see generalized mechanism for acid catalyzed hydrolysis of 2'-orthoesters under 2'-ACE Chemistry section of Technical Resources menu.) The hydrophilic 2'-ACE[®] groups ensure that any sequence or length of 2'-protected RNA is water soluble. Each oligo is accompanied by a volatile deprotection buffer and 2'-deprotection protocol. This protocol is designed to ensure complete deprotection of all 2'-hydroxyls. (Note: The 2'-deprotection is effected by a combination of pH, time and temperature. Therefore, numerous variations of these factors, including buffer, can be employed. If subsequent applications or uses make it desirable to consider alternative conditions, please contact us at the number below to discuss specific needs.)

The entire lot of RNA may be 2'-deprotected when ready for use. Alternatively, one may elect to deprotect only what is needed and store the remainder at -20°C. This will ensure that any unused RNA remains more stable over a longer period of time. Each RNA oligo that is shipped has been split into multiple tubes for this reason, and can be further split into smaller aliquots if desired. Simply, redissolve the RNA pellet in sterile water, aliquot as desired, and evaporate to dryness. The protocol can then be followed with the amount of deprotection buffer adjusted proportionately. [If additional buffer is required, please contact us at the number below.] Alternatively, the buffer can be made using the following protocol: Acetic acid (2.86 mL, 0.050 moles, 100 mM solution) is first added to sterile water (496 mL). To this is added 50-100 microliter aliquots of tetramethylethylenediamine (TEMED) to adjust the pH to 3.80. A total of 800-900 microliters of TEMED is generally required.]

After 2'-deprotection, the RNA is in 100 mM Acetate-TEMED buffer, pH 3.8. The buffer may be dried down by lyophilization or by use of a Speed-Vac to ensure no loss of product. Standard desalting techniques, e.g. ethanol precipitation, reversed-phase desalting cartridges or gel filtration (e.g. G25 sephadex), can also be used but will result in some loss of product. If the RNA is to be purified immediately after deprotection, one may minimize handling by directly loading it onto an HPLC or a polyacrylamide gel (PAGE) with loading buffer.

Contact Information

For technical questions regarding the use of siRNA reagents, please contact Dharmacon Products Technical Support at:

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