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TAMRA NHS ESTER

INTRODUCTION

Because rhodamine derivatives are generally not sufficiently stable to survive conventional deprotection, these can be attached to amino-modified oligonucleotides using post-synthesis labelling techniques. This product is the activated carboxylate, N-hydroxysuccinimide (NHS) ester, of tetramethyl rhodamine (TAMRA) dye in solution in dimethyl sulfoxide (DMSO) and is conjugated with a amino-modified oligonucleotide in sodium carbonate/bicarbonate buffer at pH 9. Although this technique is time consuming and places demands on the final purification to remove unconjugated dye, it is nevertheless routine and successful.

USE OF TAMRA NHS ESTER

Diluent: ~0.17M Solution in Anhydrous DMSO

Coupling: Conjugate with an amino-modified oligo in sodium carbonate/bicarbonate buffer (pH=9).

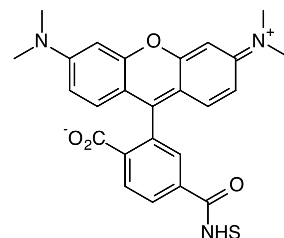
Storage: Freezer storage, -10 to -30°C, dry

LABELLING PROTOCOL

For a 0.2 µmole synthesis of an amino-modified oligo:

1. Dissolve oligo in 500 µL of conjugation buffer.
2. Add 6 µL of TAMRA/DMSO solution (~ 6 fold excess).
3. Vortex mixture and incubate at 37 °C in the dark for 1-2 hrs.
4. Separate oligo-TAMRA conjugate from salts and free TAMRA by size exclusion on a Glen Gel-Pak™ desalting column or equivalent.

***FIGURE 1:* STRUCTURE OF TAMRA NHS ESTER**



Catalog Number: 50-5910-xx

DESALTING USING A NAP-10 COLUMN

5. Equilibrate NAP column with approximately 10 mL of 50 mM TEAA buffer pH 7.
6. Load reaction mix on column and let flow into the column.
7. Add 0.5 mL TEAA buffer and let flow into column.
8. Elute oligo TAMRA conjugate with ≤ 1.5 mL TEAA buffer.
9. Collect the fast-running conjugate band and dry it in a vacuum concentrator.
10. Conjugate may be further purified by RP HPLC or PAGE to separate labelled from unlabelled oligonucleotides.