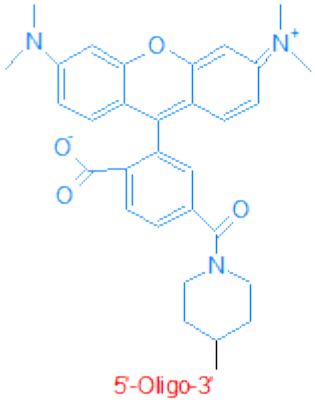
Tamra-3'

Category	Fluorescent Dyes
Modification Code	Tamra-3
Reference Catalog Number	26-6451
5 Prime	I
3 Prime	Y
Internal	I
Molecular Weight(mw)	527.53



3'-TAMRA (Carboxytetramethylrhodamine) [26-6451-XX]

Carboxytetramethylrhodamine (TAMRA) is a fluorescent dye that is a derivative of rhodamine, and is used to label oligonucleotides at the 5' or 3' ends, or internally. TAMRA has an absorbance maximum of 565 nm and an emission maximum of 580 nm. TAMRA-modified oligonucleotides play a particularly important role in both fluorescence resonance energy transfer (FRET) and real-time PCR applications.

FRET is a distance-dependent interaction between two dye molecules in which excitation is radiationlessly transferred from one dye (the donor) to the second dye (the acceptor), due to spectral overlap. Because the efficiency of the energy transfer is extremely sensitive to the distance between the molecules (varying as the inverse sixth power of that distance) (1), FRET can be used to study biological phenomena that produce changes in molecular proximity (2). For oligonucleotides slated for use in FRET application, a common donor-acceptor pair is 6-FAM (donor) / TAMRA (acceptor), due to their good spectral overlap. As the donor, 6-FAM is excited at 492 nm and transfers this energy to TAMRA, which then emits light at 580 nm. FRET oligo probes are widely used to monitor biochemical reactions, particularly in in vivo studies (3).

Besides being used as a FRET fluorophore, TAMRA also can be used as a FRET-based quencher moiety in real-time PCR probes such as TaqMan probes (4), Scorpion primers (5) and Molecular Beacons (6). For such probes, 6-FAM is used as the reporter moiety, and its emission at 521 nm is monitored. When 6-FAM and TAMRA are in close proximity, the former's fluorescence at 521 nm is quenched by the latter. After sufficient spatial separation of the two dyes during the course of the assay, 6-FAM's fluorescence is no longer quenched, and its fluorescence signal becomes observable.

TAMRA also can be used to label DNA oligos for use as hybridization probes in a variety of in vivo and in vitro research or diagnostic applications, as well as for structure-function studies of DNA, RNA, and protein-oligonucleotide complexes. Oligos labeled with TAMRA at the 5' end can be used as PCR and DNA sequencing primers to generate fluorescently-labeled PCR, sequencing or genetic analysis (AFLP or microsatellite) products.

Note that, because TAMRA is in the form of an NHS ester, the oligo first must be synthesized with an Amino C6 Linker (for the ends) or the Amino C6 version of the base phosphoramidite (for internal labeling). The TAMRA-NHS ester is then manually attached to the oligo through the amino group in a separate reaction post-synthesis.

References

1. Stryer, L., Haugland, R.P. Energy transfer: a spectroscopic ruler.*Proc. Natl. Acad. Sci. USA* (1967), **58**: 719-726.
2. Wu, P., Brand, L. Resonance energy transfer: methods and applications.*Anal. Biochem.* (1994), **218**: 1-13.
3. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications.*Biotechniques* (2001), **31**: 1106-1121.
4. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization.*PCR Methods Appl.* (1995), **4**: 1-6.
5. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
6. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* (1996), **14**: 303-308.