4,5-dichloro-dimethoxy-fluorescein (JOE) is a dichlorinated, dimethoxylated version of the fluorescent dye fluorescein, and is used for labeling oligonucleotides at either the 5'- or 3'-end, or internally (via an NHS ester). JOE has an absorbance maximum of 520 nm and an emission maximum of 548 nm. JOE can be used in real-time PCR applications as a reporter moiety in TaqMan probes (1), Scorpion primers (2) and Molecular Beacons (3). For such probes, JOE is most commonly paired with the dark quencher BHQ-1, as the two have excellent spectral overlap. JOE-labeled primers have also been used for bacterial SNP genotyping by allele-specific real-time PCR (4).

JOE 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 JOE 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: If JOE is on the 3'-end of the oligo, it cannot be used as a primer in PCR-based applications.

Note that for internal labeling of an oligo, the NHS ester form of JOE must be used. Consequently, the oligo first must be synthesized with the Amino C6 version of the base phosphoramidite (for example, Amino-C6-dA, Amino-C6-dC, etc.) at the desired position to be labeled with JOE. The appropriate JOE-NHS ester is then manually attached to the oligo through that base's C6 amino group in a separate reaction post-synthesis. References