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## 5'-DICHLORO-DIMETHOXY-FLUORESCIN PHOSPHoramidite

### INTRODUCTION

5'-Dichloro-dimethoxy-fluorescein is more commonly known as JOE™ to those familiar with the dye sets used in Applied Biosystems DNA PRISM sequencers - e.g., Dye Set 20 and 32, which contain FAM/JOE/TAMRA/ROX and FAM/JOE/NED/ROX, respectively.

As a dye, 5'-dichloro-dimethoxy-fluorescein gained popularity because its emission is nicely resolved from both FAM and TAMRA, falling exactly between them. This allows multiplex detection without too much signal bleed through into other channels, making it extremely useful in automated DNA sequencing. In addition, because of the electron-withdrawing groups on the xanthenone ring, the 5'-dichloro-dimethoxy-fluorescein dye is less prone to quenching due to protonation. As such, its fluorescence is much less pH sensitive than its popular cousin, fluorescein. Even at pH 5, our in-house testing indicates 5'-dichloro-dimethoxy-fluorescein's signal dropped by only 30%.

With its high extinction coefficient of 75,000 L/mol.cm, a quantum yield of fluorescence of 0.581, and excellent stability to standard deprotection conditions in ammonium hydroxide, 5'-dichloro-dimethoxy-fluorescein has been a popular addition to our ever-expanding selection of fluorophore phosphoramidites.

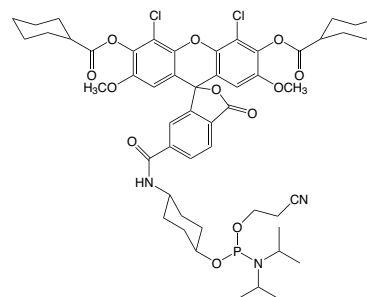
This product, (1) in Figure 1, has been available from Glen Research since 2008. Beginning in 2012, due to its increasing popularity, we have been evaluating options for scale up of the current product. Due to several factors, we have concluded that synthesis of this product on a larger scale is not feasible and we have decided to replace it with the optimized product described below.

### 5'-DICHLORO-DIMETHOXY-FLUORESCIN II

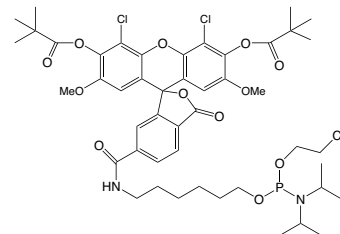
This improved version of 5'-dichloro-dimethoxy-fluorescein phosphoramidite (2) is shown in Figure 1. This second generation version uses the identical chromophore for 5'-dichloro-dimethoxy-fluorescein but more standard pivaloyl protecting groups are used to protect the fluorophore during synthesis. The spacer used is also the more standard C6 spacer.

Having implemented these changes, we feel confident that we are in a much better position now to support future growth of 5'-dichloro-dimethoxy-fluorescein sales with much better synthesis scale and consequent pricing profile.

FIGURE 1: PHOSPHoramidite STRUCTURES



(1) 10-5904: 5'-Dichloro-dimethoxy-fluorescein Phosphoramidite



(2) 10-5906: 5'-Dichloro-dimethoxy-fluorescein II Phosphoramidite

### KEY POINTS

- 5'-Dichloro-dimethoxy-fluorescein fluorophore unchanged.
- Protecting groups and spacer standard.

### USE OF 5'-DICHLORO-DIMETHOXY-FLUORESCIN II

**Diluent:** Anhydrous Acetonitrile

**Coupling:** 6 minute coupling time recommended.

**Deprotection:** Use Ammonium Hydroxide and deprotect as required by nucleobases. If AMA is used, a small amount of a non-fluorescent impurity will be formed. To eliminate this impurity, first deprotect with ammonium hydroxide for 30 minutes at room temperature, add an equal volume of 40% methylamine and then complete the deprotection as required by the nucleobases - e.g., 10 minutes at 65°C or 2 hours at room temperature for standard bases.

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

**Stability in Solution:** 1-2 days

### Spectral Characteristics

E260	12,000 L/mol.cm		
Abs. Max.	525 nm	Emax	75,000 L/mol.cm
Emission Max.	548 nm	QY	0.58