User Bulletin: ABI 392/4 Nucleic Acid Synthesizers

Subject: FastPhoramidite Reagents for DNA Synthesis with Rapid Deprotection at All Scales

User Bulletin - Number 85

All DNA/RNA Synthesizers

FastPhoramidite Reagent Use

This User Bulletin announces a new set of reagents, FastPhoramidites, for the synthesis of high purity and high yield oligodeoxynucleotides, with complete deprotection in one hour at 65 °C. FastPhoramidite reagents (dA ^{bz}, dG^{dmf}, dC^{bz}, T) are distinguished from standard reagents only by the protecting group on the G monomer. Because isobutyryl (ibu) on G is the slowest protecting group to be removed, simple replacement with the much more labile dimethylformamide (dmf) results in very rapid deprotection.

FastPhoramidite reagents are restricted to the rapid production of 50-mers and shorter. A new iodine formulation is required for optimum results with FastPhoramidite reagents. All scales, 40 nmol, 0.2 µmol, 1 µmol, and 10 µmol, are provided with FastPhoramidite supports. All models of Applied Biosystems DNA/RNA synthesizers may use FastPhoramidite reagents.

Rapid deprotection of synthetic oligodeoxynucleotides has been accomplished with more labile protecting groups, [1] such as the FOD (Fast Oligonucleotide Deprotection) reagents,[2] alternative deprotection reagents,[3] and elevated temperatures.[4] The FOD reagents (dA^{dmf} , dG^{dmf}, dC^{ibu}, , and T) were introduced in 1990 and are useful for short primers and probes (smaller than 35-mers).

Our FastPhoramidite reagent set is configured with the standard A,C, and T reagents, and the proven dG^{dmf} reagent. Deprotection requires one hour at 65 °C. Compared to FOD reagents, FastPhoramidite reagents give improved synthesis performance.[5] For these reasons, we are discontinuing FOD reagents.

Using FastPhoramidite Reagents

- FastPhoramidite reagents are used with the same protocols and procedures as standard reagents.
- No Synthesis Cycle, Begin Procedure, Bottle Change Procedure, or End Procedure modifications are necessary.
- FastPhoramidite reagents may be dissolved by the AutoDilute Procedures (Models 392 and 394), or the manual dilute protocol.
- FastPhoramidite reagents do not require special handling. Store the phosphoramidites and supports at room temperature in a dry environment up to one year.
- Like standard phosphoramidites, FastPhoramidite dGdmf has a useful

lifetime on the synthesizer in acetonitrile solution of two weeks (0.2 µmol, 1 µmol, 10 µmol scale synthesis), and three weeks (40 nmol scale synthesis).

New Iodine Formulation

A new iodine formulation provides improved oligonucleotide purity, especially with FastPhoramidite reagents. The new formulation is provided in the regular bottle sizes and is placed in the regular bottle position 15 on the synthesizer. No cycle modifications or other changes are necessary. This new reagent has less iodine, more water, and the same amount of tetrahydrofuran and pyridine as the previous iodine formulation. Complete and rapid oxidation is realized at all scales, for RNA synthesis, and for standard phosphoramidites and FastPhoramidites. The iodine reagent can be stored up to six months in the refrigerator (4 °C) or up to six weeks on the synthesizer.

Deprotection Conditions

- **For Models 394, 392, 380B, and 380A:** Cleave oligonucleotides made with FastPhoramidite by the usual, approximately one-hour End Procedures.
- **For the 391 PCR-MATE, and the Model 381:** Cleave by the manual, double-syringe method with concentrated ammonium hydroxide (J.T. Baker Co., P/N 9072-01).
- After cleavage, deprotection is conducted in the standard 4 mL screw cap glass vials (Applied Biosystems P/N 400048) with Teflon-lined caps (Wheaton, Inc. P/N 240408, size 13-425).
- Oligonucleotides made with FastPhoramidite reagents are fully deprotected after one hour at 65 °C, or two hours at 55 °C. Room temperature deprotection is not advised since a small amount of protected nucleobases is still observed after 24 hours at room temperature in concentrated ammonium hydroxide.
- The support material should not be transferred from the synthesis column to a vial and heated in ammonium hydroxide for a combined cleavage/deprotection operation. Such a procedure will dissolve CPG and liberate impurities which remain bound to the support during the one hour, room temperature cleavage step.

Synthesis Limits and Specifications with FastPhoramidite Reagents

We recommend FastPhoramidite reagents for the synthesis of oligonucleotides up to 50 bases in length. For longer oligonucleotides, which require greater stability, we recommend standard dGibu reagents.

FastPhoramidite reagents will give the same high synthesis efficiency as standard phosphoramidite reagents. For a typical 20-mer, expect crude yields of at least 5 optical density units (ODU) at 40 nmol, 20 ODU at 0.2 µmol, 100 ODU at 1 µmol, and 800 ODU at the 10-µmol scale (see 4-33 of the 392/394 User's Manual).

Following the recommended deprotection conditions, no base modifications or incomplete deprotection should be detectable by enzymatic digestion/HPLC analysis.[6] Oligonucleotides made with FastPhoramidite reagents can be analyzed and purified by conventional methods.[6]

WARNING

Considerable pressure results from heating enclosed concentrated ammonium hydroxide solutions. Deprotection in concentrated ammonium

hydroxide in glass vessels above 65 °C is considered unsafe, with a demonstrated higher frequency of rupture. Safety glasses with side-shields should be worn by all personnel in a laboratory with synthesizers or deprotection stations. The heating block or water bath used for deprotection should be placed behind a plexiglass shield. Hot deprotection vials should be handled behind a shield and gloves should be worn. After deprotection, the vials should be chilled on ice to reduce pressure and facilitate pipette transfer.

Ordering FastPhoramidite Reagents

For easy reference when ordering, the products mentioned in this User Bulletin are listed below:

P/N	Phosphoramidites	Quantity
401181	FastPhoramidite dG^{dmf}	250 mg
401182	FastPhoramidite dG^{dmf}	500 mg
401183	FastPhoramidite dG^{dmf}	1 g
401165	FastPhoramidite dG^{dmf}	2 g (60-mL bottle)
Synthesis Columns		
401347	40-nmol Polystyrene dG^{dmf}	each
401184	0.2-µmol CPG dG^{dmf}	each
401346	1-µmol CPG dG^{dmf}	each
401348	10-µmol CPG dG^{dmf}	each
Iodine Reagents		
401732	Iodine (0.02 M)	200 mL
401632	Iodine (0.02 M)	450 mL

References

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3. Polushin, N.N., Morocho, A.M., Chen, B-C., and Cohen, J.S. "On the Rapid Deprotection of Synthetic Oligonucleotides and Analogs," (1994) *Nucleic Acids Res. 22*, 639-645. Polushin, N.N., Pashkova, I.N. and Efimov, V.A. International Symposium, "Synthetic Oligodeoxynucleotides: Problems and

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6. *Evaluating and Isolating Synthetic Oligonucleotides*, (1992) Applied Biosystems, Foster City, California.
