

USER BULLETIN

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SUBJECT: Analysis of the Oligonucleotide Purification Cartridge (OPC) Detritylation Procedure

Data from our R&D staff show that a relatively slow reagent flow rate of 1 to 2 drops per second is essential to consistent results with OPC. This is especially true of the detritylation step with 2% (aq) trifluoroacetic acid (TFA). Our data show that a detritylation time of at least two minutes is optimal for the support-bound reaction.

Tests show that this amount of acid treatment to the OPC-bound product does *not* result in any apparent depurination to the unprotected bases. The following set of data shows HPLC base composition analysis¹ of a model oligonucleotide, d[A(T)₁₆C], exposed to the TFA solution on OPC. Table 1a shows the results after the suggested two minute exposure to TFA. Table 1b shows the results after one-hour TFA exposure. In a control analysis (Table 2), the compound was purified by PAGE and only exposed to the conventional trichloroacetic acid solution and acetic acid. Compared to the control analysis, the TFA treatment showed no significant drop in the amount of adenosine nucleoside present in the product.

These data clearly demonstrate that depurination is not an issue with the use of the TFA solution. Also, no other modified nucleosides were detected by the base composition assay. This indicates there is no inherent problem with chemical homogeneity regarding the use of OPC.

Table 1a. After two-minute treatment with TFA on OPC.

Base	Area %*	Number of bases	
		Actual	Theoretical
dC	5.81	1.04	1
dG	0.00	0.00	0
dT	88.66	15.95	16
dA	5.51	0.99	1

Table 1b. After one-hour treatment with TFA on OPC.

Base	Area %*	Number of bases	
		Actual	Theoretical
dC	5.81	1.04	1
dG	0.00	0.00	0
dT	88.85	15.99	16
dA	5.33	0.95	1

Table 2. Gel-purified results (control)

Base	Area %*	Number of bases	
		Actual	Theoretical
dC	5.77	1.03	1
dG	0.00	0.00	0
dT	88.73	15.97	16
dA	5.49	0.98	1

* corrected for extinction coefficients

1) Eadie, J.S., L.J.McBride, J.W.Efcavitch, L.B.Hoff and R.Cathcart, (1987) High-Performance Liquid Chromatographic Analysis of Oligodeoxyribonucleotide Base Composition, *Anal. Biochem.* 165, 442-447.