### Waters™



# DMT On Purification of DNA Oligonucleotides <35mer Using Oasis HLB SPE Products

**Waters Corporation** 

This is an Application Brief and does not contain a detailed Experimental section.

#### **Abstract**

This application brief highlights on purification of DNA using Oasis HLB SPE products.

#### Introduction

Capillary gel electrophoresis analysis of fractions from oligodeoxythymine (30-mer) SPE purification.

#### Experimental

OASIS® HLB EXTRACTION METHOD		96-well plate 30 mg WAT058951	3 cc cartridge 60 mg WAT094226	6 cc cartridge 200 mg WAT106202	
STEP	SYNTHESIS SCALE	0.1-0.2 μmol	0.2 µmol	0.1-1 µmol	
1 CONDITION: ACN	Organic solvent wets (conditions) the sorbent and frits	1 mL	2 mL	2 mL	GRAVITY FLOW
2 EQUILIBRATION: 0.1 M TEAA, pH 7	Removes ACN and equilibrates sorbent with TEAA	1 mL	2 mL	2 mL	
3 SAMPLE LOAD: in 0.1 M TEAA, pH 7	Retains target oligonucleotide and failure sequences	1 mL	2 mL	3 mL	
4 WASH 1: 8% ACN in 0.1 M TEAA, pH 7 (v:v)	Remove weakly retained failure sequences	1 mL	2 mL	3 mL	ml/min
5 WASH 2: 12% ACN in 0.1 M TEAA, pH 7 (v:v)	Remove strongly retained failure sequences	1 mL	2 mL	3 mL	FLOW 1-2 m
6 DETRITYLATION: 2% TFA Apply half of the volume by vacuum, release vacuum, wait 1 min, then resume vacuum	On cartridge cleavage of DMT group from target oligonucleotide	1 mL	2 mL	3 mL	
7 ELUTION: 20% ACN in 0.36 M TEAA, pH 11.3	Neutralizes TFA, dissolves and elutes target oligonucleotide	1 mL	2 mL	2 mL	VACUUM

## 0.1 M TEAA, pH 7 Buffer – commercially available For 100 mL of 0.36 M TEAA buffer:

Mix 94.5 mL of MilliQ water and 0.5 mL of glacial acetic acid.

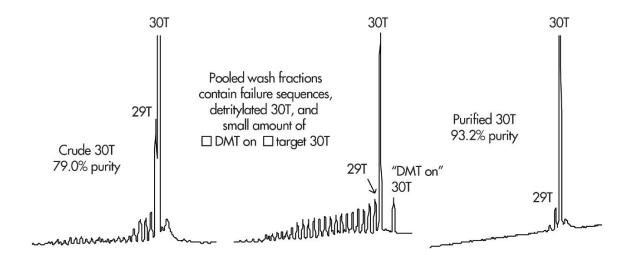
While mixing slowly add 5 mL of TEA, mix until it dissolves.

pH of final 0.36 M solution is approximately 11.3 (desirable values are between 10.8-11.5)\*

\* Keep in closed polypropylene bottle. Handle in hood, TEA has a strong odor.

#### **Results and Discussion**

## Capillary Gel Electrophoresis Analysis of Fractions from Oligodeoxythymine (30-mer) SPE Purification



#### Troubleshooting

Flow rates of >0.5 mL/min in the load step (step 3) will cause sample breakthrough which reduces oligonucleotide recovery in final elution (step 7).

#### **Recovery Calculation**

Recovery of target oligonucleotide is determined by analysis with a UV absorbance spectrometer.

Take 10  $\mu$ l of sample solution (prior to loading), dilute to 1mL and measure Absorbance A<sub>260</sub>(L).

Take 10  $\mu$ L of final elution (step 7), dilute to 1 mL and measure absorbance A<sub>260</sub>(E).

 $V_E$  = elution volume from step 7

 $V_L$  = elution volume from step 3

Recovery (%) = 
$$\left[\frac{A_{260}(E)}{A_{260}(L)} \times 100\right] \times \frac{V_E}{V_L}$$

DMT = dimethoxytrityl

ACN = acetonitrile

TEAA = triethylamine acetate

TFA = trifluoro acetic acid

Oligonucleotide Purity Determined by Capillary Gel Electrophoresis.

#### References

1. M. Gilar, E.S.P. Bouvier, J. Chromatography A, vol 890 (1), 167-177.

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