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アプリケーションノート

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	VACUUM FLOW 1–2 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	
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	

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.

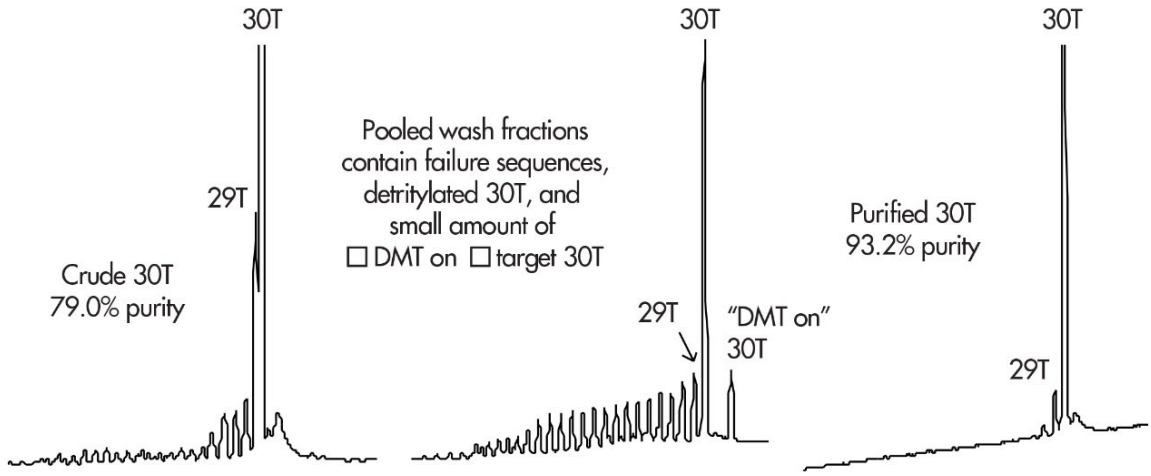
While mixing slowly add 5 mL of TEA, mix until it dissolves.

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 µl of sample solution (prior to loading), dilute to 1mL and measure Absorbance A_{260}(L).

Take 10 µL of final elution (step 7), dilute to 1 mL and measure absorbance A_{260}(E).

V_E = elution volume from step 7

V_L = elution volume from step 3

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

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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