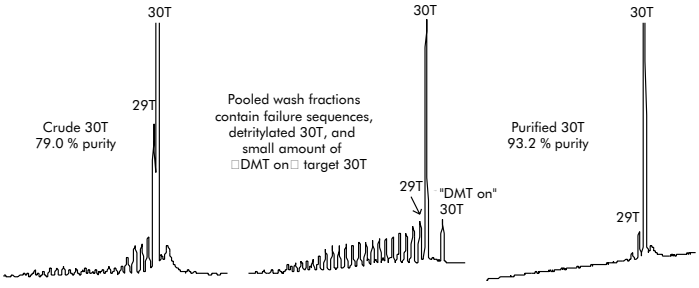
"DMT ON" PURIFICATION OF DNA OLIGONUCLEOTIDES < 35 MER USING OASIS® HLB SPE PRODUCTS

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	

CAPILLARY GEL ELECTROPHORESIS ANALYSIS OF FRACTIONS FROM OLIGODEOXYTHYMINE (30-mer) SPE PURIFICATION



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.

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.

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