

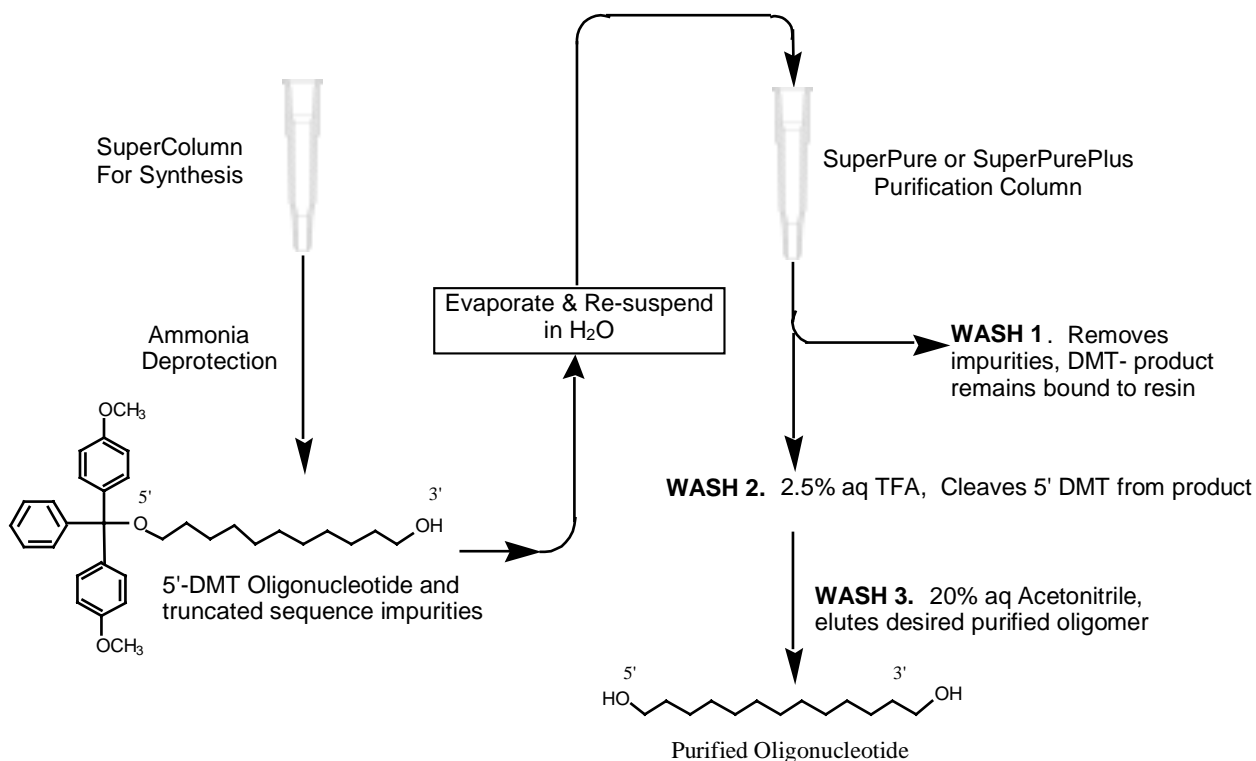
PRODUCT USE NOTE

SuperPure™ and SuperPure Plus™ Column DNA Purification

Oligonucleotides (whether synthetic or natural) may be purified by a number of techniques, including polyacrylamide gel electrophoresis and high performance liquid chromatography (either by ion exchange or reverse-phase methods). Synthetic DNA can be conveniently de-salted and purified by reverse-phase low-pressure separation on BTI's SuperPure (SP-1000) and SuperPure Plus (SP-2000) columns (Figure 1).

BTI's SuperPure Columns are intended for reverse-phase purification of 5'-DMT oligonucleotides followed by on-column detritylation. The method relies on strong binding between the 5'-DMT protected product and a hydrophobic optimized polymer packing (polystyrene), thus allowing separation from truncated sequences which, due to a lack of DMT group, do not bind. Treatment with acid (**WASH 2**) removes the DMT group from the product, the desired product (free from organic residues) is eluted with 20% acetonitrile (**WASH 3**). The method is rapid, efficient and permits many samples to be purified simultaneously. The SuperPure column has capacity to handle crude, cleaved DNA from a 50 nmol scale synthesis. Crude DNA from a 200 nmol synthesis scale can be loaded on the SuperPure Plus. The purified yield will depend, in each case, on sequence length and the quality of the crude sample.

Fig. 1—Schematic Outline of Oligo Purification on the **SuperPure & SuperPure Plus** Columns



SUGGESTED WORK-UP PROTOCOL

Note: SuperPure Column Volume = ~200µl; SuperPure *Plus* Column Volume = ~500µl

1. Program DNA synthesizer to leave terminal 5'-DMT group attached;
2. Cleave and deprotect product with conc. aqueous ammonia (5 hours at 55°C with standard deprotection procedure);
3. Evaporate cleavage solution to dryness;
4. Re-suspend in 0.2 ml (SuperPure) or 0.5ml (SuperPure *Plus*) 0.1N TEAA or H₂O;
5. Pre-equilibrate column by passing 2 column volumes (CVs) of acetonitrile through the column;
6. Repeat with 3 CVs 1N triethylamine acetate solution (TEAA);
7. Take the oligo solution of step 4 and apply slowly to the column while collecting effluent in a separate micro-centrifuge tube. Reload effluent and pass once more through column (optional);
8. Pass 3 CVs of 2.8% ammonia solution slowly through the column;
9. Wash by slow passage of 2 CVs water (Figure 1, WASH 1);
10. Pass 2 CVs of 2.5% TFA through the column, wait 5 minutes;
11. Pass 3 or more CVs of water through column (Figure 1, WASH 2). Check to see pH is neutral.
12. Slowly pass 2 CVs 20% acetonitrile through the column, collecting effluent containing the desired purified product in a labeled microcentrifuge tube (Figure 1, WASH 3).
13. Speedvac™ the solution to dryness, analyze and use as desired.

Step No	Reagent Name	Approximate SuperPure	Volume, µL SuperPure <i>Plus</i>	Repeat "n" times	Purpose	Notes
1					End synthesis with 5'-DMT ON	
2	NH ₄ OH (aq)	200	500	1X	Cleave & Deprotect	5 hours at 55°C
3	SpeedVac™			1X	Evaporate to dryness	
4	H ₂ O (or 0.1N TEAA)	200	500	1X	Re-suspend	Re-dissolves Oligo
5	MeCN	200	500	2X	Rinse column	
6	1N TEAA	200	500	3X	Activate Column	
7	Add Crude DMT-Oligo			1X	Load Oligo	
8	2.8% NH ₄ OH	200	500	3X	Elute Failures	
9	H ₂ O	200	500	2X	H ₂ O Rinse	
10	2.5% TFA	200	500	2X	Deblocks DMT	Wait 5 minutes; Note Orange color
11	H ₂ O	200	500	Min. 3X	Removes TFA	Effluent should be neutral
12	20 % MeCN in H ₂ O	200	325	2X	Elutes pure oligo	Insert collection tubes
13					Speedvac	

REAGENTS

20% acetonitrile in water (v/v) may be made by appropriate dilution (e.g. 20 mL acetonitrile diluted to 100 mL). Perform in fume hood with eye and hand protection.

1N TEAA In a 250 mL bottle (e.g. VWR, Cat. No. 16159-856) equipped with magnetic stir bar, place water (75 mL) and add glacial acetic acid (5.7 mL), stir until completely mixed. SLOWLY add with continuous mixing triethylamine (13.9 mL, ALDRICH 23, 962-3; do not use if at all colored!). Add water to 100 mL total volume. Check that pH is neutral. Perform in fume hood, with eye and hand protection.

0.1N TEAA Solution: Dilute above one to ten

2.5% TFA solution. Place 97.5 mL water and stir bar in 250 mL bottle. Add 2.5 mL TFA (Aldrich T6,220-0) slowly with stirring. Perform in fume hood with eye and hand protection. Neat TFA is a corrosive, toxic irritant!!! In event of any skin contact wash with dilute sodium bicarbonate solution.

2.8% ammonium hydroxide in water. Use same precautions as for TFA. Add 10 mL concentrated ammonia (d = 0.88) to 90 mL of water.

FURTHER POINTERS

- TEAA equilibration improves performance and capacity, poor results will be obtained if this step is eliminated.
- Do not overlook the 2 CVs H₂O wash in step 9.
- Remember to clearly label each SuperPure Column and collection tube

ORDERING INFORMATION

Part No.	Description	Unit	Price¹
SP-1000-1	SuperPure™ Column (for ~50 nmol scale synthesis)	each	\$1.75
SP-1000-96	SuperPure™ Column (for ~50 nmol scale synthesis)	96	\$150
SP-2000-1	SuperPure <i>Plus</i> ™ Column (for ~200 nmol scale synthesis)	each	\$2.00
SP-2000-96	SuperPure <i>Plus</i> ™ Column (for ~200 nmol scale synthesis)	96	\$170.00

¹ Prices as of 7.30.03. Please check our website or call Biosearch Customer Care at 1.800.436.6631 for current pricing.