

- Place the assembly into a centrifuge with 96-well plate-carrier rotor and centrifuged at 1,000 $\times$ g for 2 minutes to remove the storage buffer. Discard the flow-through.
- Rinse the wash plate three times with deionized water, dry and save for future use.
- Stack the desalt plate on top of a sample collection plate (blue), aligning the alphanumeric indices on the plates.
- Apply sample (20-100 $\mu$ l) to the center of the resin bed. To expel the entire sample, carefully touch pipette tip to the resin. For 20  $\mu$ l oligo samples (>300  $\mu$ g/ml), apply a 20  $\mu$ l stacker of water or buffer on top of resin bed after the sample wash has fully absorbed to ensure maximal protein recovery.
- Centrifuge the plate assembly at 1,000 $\times$ g for 2 minutes to collect the desalt sample. Discard the desalt plate or reserve it for further balancing purpose.

## ZARA Size exclusion cartridges/plates

Gel filtration removes lower molecular weight molecules, inorganic salts and very short failure sequences. Purification occurs by passing a sample through Sephadex column, which separates molecules based on size.

ZARA Size exclusion 8Z is recommended for oligo with molecular weight > 8,000; For molecular weight > 30,000, we recommend ZARA size exclusion 30Z products.

### General protocol:

- Oligo sample preparation: use amino incubation solution of synthesized CPGs
- Place the plate over appropriate wash plates and place it into a centrifuge with correct carrier.
- Prepare plate: Centrifuge for 1 minute at 750  $\times$ g to remove residual buffer. Discard the wash plates containing excess water.
- Loading Oligo solution: Carefully and rapidly load 50  $\mu$ l sequencing reaction mixture before the gel dries out.
- Eluting Oligo: Centrifuge for 2 minutes at 750  $\times$ g to eliminate big dye terminators, salts, and low molecular weight species.
- Purified DNA of the sequencing reaction is recovered in collection plate

## Large Scale Oligo Purification Service

Using ZARA FLASH-DEP kit, Orochem provides high efficiency large scale process for the cleavage, deprotection and recovery of the newly synthesized Oligonucleotide under organic conditions and performed at room temperature.



## Oligo Purification Kits: A Path to Purity

Discover our range of Oligo Purification Kits for precise and efficient purification of oligonucleotides. Orochem offers both reversed-phase and size exclusion purification products. All products are sold in spin column, 96-well plate and 384-well plate formats.



### USA HQ

Tel: 630 210 8300  
info@orochem.com  
www.orochem.com

340 Shuman Blvd  
Naperville, IL 60563  
USA

### INDIA

Tel: +91-22-27603569  
orochemindia@orochem.com  
www.orochem.com

Plot # R 588/1, TTC Indl.  
Area Rabale Navi Mumbai  
400 705 India

For Free Consultation, Please Enquire:

Dr. Anil Oroskar, PhD  
Dr. Asha Oroskar, PhD

Purify with Precision, Excel in Research.

## Product Selection Guide

Oligo					
	Reverse Phase		Size Exclusion		
Oligo MW	< 7K		> 7K	> 8K	> 30K
Oligo Sample	Aqueous Sol				
	Neutralized				
Product	C8 or C18	OPC	Zara Desalting	Zara Size Exclusion	
			8Z		30Z

### Reversed-Phase Purification Products

#### Orpheus C8 and C18 Cartridges/plates

Desalting procedures remove inorganic salts, traces of organic compounds, low molecular weight impurities, and short failure sequences. Desalting is accomplished by taking advantage of size (molecular weight) or solubility differences. Aqueous samples should be neutralized before loading to the product

- Aqueous samples (in neutral pH)
- Wide range of oligo with different molecular weight
- High purity product

#### General protocol: (30 mg C18 cartridge)

- Condition the C18 product by passing 0.5 ml of acetonitrile through it, followed by 0.5 ml of deionized water.
- Load 0.1 ml of neutralized oligo solution. Pass the oligo solution slowly through the cartridge. Collect flow-through in the same tube.
- Wash cartridge slowly with 0.5 ml deionized water.
- For short oligos (less than 40 mer): Wash cartridge slowly with 0.5 ml of 60% methanol (HPLC grade or better). Collect the eluate.

For long oligos (greater than 40 mer): Wash cartridge slowly with 0.5 ml of 70% acetonitrile (HPLC grade or better). Collect the eluate.

### Reversed-Phase Purification Products

#### OroPure OPC cartridges/plates

OroPure products are polymer based reversed-phase products. Compared to C8 or C18 products, they are stable to organic and basic samples.

These products can be used to purify oligo in general or to purify DMT-on oligo. The principle is the OPC product retains the dimethyltrityl (DMT) group of the trityl-on oligonucleotide while non-DMT bearing failure sequences, by-products, and other impurities wash through. The DMT group is removed with mild acid, allowing the purified, detritylated oligonucleotide to be eluted. This product not only eliminates salts and trace organic impurities from the crude oligonucleotide, but also provides pure oligonucleotides for sequencing primers, PCR primers, hybridization probes, and gene constructions.

- Organic samples
- Wide range of oligo with different molecular weight
- High purity product
- Able to separate DMT-on oligo from other impurities

### General protocol of purification DMT-on oligo:

The flow rate of solvents through the cartridge should be regulated at rate of 1-2 drops/sec. (30 mg OPC cartridge)

- Oligo sample preparation: use amino incubation solution of synthesized CPGs
- Condition the OPC product with 2 x 0.5 mL of methanol, followed by 0.5 mL of CAN, then 2 x 0.5 mL 2.0 M TEAA.
- Load the ammonium incubation mixture (0.1 mL, NH<sub>4</sub>OH or AMA mixture). Collect the flow-through
- Wash the OPC with 2 x 0.5 mL of ammonium hydroxide:water (1:20).
- Wash the OPC with 0.5 mL of DI water
- Wash OPC with 3-5% of ACN/water (removing non-DMT bearing oligo)
- Detritylate support bound oligonucleotide with 0.5 mL of 2% TFA: a faint pink/orange band may be observed in the cartridge (good evidence of the presence of the oligonucleotide!)
- wash the OPC with 0.5 mL of water.
- Elute oligonucleotide from OPC with 0.1 mL of 30% ACN/water. Collect the eluted fraction.

### General protocol of purification oligo from other impurities:

The flow rate of solvents through the cartridge should be regulated at rate of 1-2 drops/sec.

- Oligo sample preparation: use amino incubation solution of synthesized CPGs
- Condition the OPC product with 2 x 0.5 mL of methanol, followed by 0.5 mL of CAN, then 2 x 0.5 mL 2.0 M TEAA.
- Load the deprotection mixture (0.1 mL, NH<sub>4</sub>OH or AMA mixture). Collect the flow-through
- Wash the OPC with 2 x 0.5 mL of ammonium hydroxide:water (1:20).
- Wash the OPC with 0.5 mL of DI water
- Detritylate support bound oligonucleotide with 0.5 mL of 2% TFA: a faint pink/orange band may be observed in the cartridge (good evidence of the presence of the oligonucleotide!)
- Wash the OPC with 0.5 mL of water.
- Elute oligonucleotide from OPC with 0.1 mL of . 30% ACN/water. Collect the eluted fraction.

### Size Exclusion Oligo Purification Products

Resin-based desalting is performed by allowing the small molecules to freely enter the resin pore and be retarded in their flow through the packed resin bed while the high molecular weight molecules like proteins or oligo in the sample are excluded from the resin and rapidly exit the column.

### ZARA desalting cartridges/plates

Orochem high performance ZARA desalting spin columns and 96-well desalting plates contains a proprietary size exclusion chromatography resin that provides excellent oligo desalting performance with high oligo recovery in a centrifuge format for 20µL - 4 mL samples. Samples containing as low as 20µg/mL of oligo can be processed with retention of salts and other small molecules (< 1000Da). The spin column method eliminates cumbersome column preparation or equilibration, allowing multiple-sample processing in 7,000 Da

#### General protocol

- Equilibrate ZARA 96-well Desalt Spin Plates to room temperature
- Remove the sealing material from the bottom of the plate and place it on top of a wash plate.
- Remove the sealing material from the top of the desalt plate.