

Oligonucleotide Buffer Exchange and Desalting Protocol

Oligonucleotide modification protocols require a buffer exchange both prior to modification and after modification. The initial desalting removes interfering amine contaminants from the synthesis process (e.g., ammonium ions), while exchanging the oligonucleotide into an optimized reaction buffer. Desalting is required after modification to remove excess unincorporated linker. Diafiltration spin columns may be used for desalting oligonucleotides greater than 5,000 Daltons, or approximately 20 nucleotides. Vivaspin™ 500 or Amicon™ Ultra (0.5 ml) diafiltration spin filters may be used. A molecular weight cutoff of 3 kDa is recommended for oligos \leq 30 nucleotides, and a 5 kDa molecular weight cutoff may be used for oligos $>$ 30 nucleotides for faster processing.

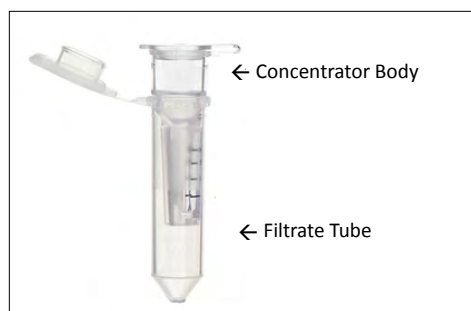


Figure 1: Diafiltration Spin Column

Materials Required:

Reagents	Equipment
Modification Buffer (10X)*	Diafiltration Spin Columns
Conjugation Buffer (10X)*	Variable-speed Bench-top Microcentrifuge
	UV-Vis Spectrophotometer or NanoDrop™

* Depending on desired buffer.

Oligonucleotide Buffer Exchange & Desalting

A. Oligonucleotide Sample Loading

- Oligonucleotide preparation.
 - For oligonucleotide in solid form:
 - Spin down the oligonucleotide (20-100 OD₂₆₀ units) to ensure the lyophilizate is settled at the bottom of the tube.
 - Dissolve the oligonucleotide in 250 μ l of buffer. 1X Modification Buffer, pH 8.0, is used for amino oligos prior to modification with an NHS ester-based linker. 1X Conjugation Buffer, pH 6.0, is used to desalt linker-modified oligonucleotide in preparation for conjugation.
 - For oligonucleotides in solution:
 - The specified diafiltration spin filters are designed to contain volumes of 500 μ l or less. If volumes greater than 500 μ l are to be processed, multiple filters or multiple loadings on the same filter will be required.

- Determine oligonucleotide concentration and amount available (optional but recommended).
 - Determine the oligo concentration and amount available using the [Oligonucleotide Concentration Determination](#) instructions.
- Add 250 μ l of the desired buffer to the stock oligonucleotide solution to bring the total volume to 500 μ l.
- Add the stock oligo solution to the diafiltration spin filter body and close the lid tightly.
- Place the apparatus into the centrifuge with an appropriate balance.

B. Oligonucleotide Desalting and Buffer Exchange

- Centrifuge the diafiltration apparatus for 7-10 minutes at 15,000 x g.

Note: Ensure the centrifuge is set to "g" or RCF rather than RPM.
- After the spin is complete, check to ensure the retentate volume is at 50 μ l or less in the concentrator body. If the volume is greater than 50 μ l, spin the column several minutes longer until the volume is \leq 50 μ l.
- Excess buffer will flow into the lower filtrate tube. Transfer the flow-through after each spin to a new microcentrifuge tube. This is a precautionary step to ensure no oligonucleotide is lost if the membrane is ruptured or damaged.
- Add 450 μ l of fresh buffer to the concentrator body and pipet up and down to mix the oligo solution. Be sure not to touch or damage the white filter membrane surface with the pipette tip.
- Centrifuge the apparatus for 7-10 minutes at 15,000 x g. Check that the retentate volume is 50 μ l or less. Spin for an additional several minutes if required to reduce the volume to \leq 50 μ l.
- Transfer the flow-through to a microcentrifuge tube.
- Repeat steps 4 to 6 an additional two times.
- After the last spin, transfer the desalted oligonucleotide from the concentrator body to a new microcentrifuge tube.
- Calculate the amount of additional buffer needed to dilute the oligo to 0.3 - 0.5 OD/ μ l.
- Add this volume of buffer to the concentrator body and rinse the membrane and walls to maximize oligo recovery.
- Briefly spin the diafiltration filter to collect the buffer, then transfer it to the desalted oligo stock solution.
- Vortex the solution gently to mix.
- Determine the concentration and amount of oligo recovered - refer to step A2 for details. The volume of oligonucleotide solution can be adjusted by adding additional buffer until the OD₂₆₀/ μ l concentration is between 0.3 to 0.5 OD₂₆₀/ μ l, if needed.