

Oligonucleotide Buffer Exchange & Desalting Protocol



Materials Required

Reagents	Catalog #	Equipment
Diafiltration spin columns	S-4004	Variable-speed bench-top microcentrifuge
Modification Buffer*	S-4003	UV-Spectrophotometer
Conjugation Buffer*	S-4002	

*Depending on desired final buffer

Oligonucleotide modification protocols require a buffer exchange & desalting of amino-oligonucleotide. Desalting removes small, interfering amine contaminants from the sample while exchanging the oligonucleotide into specially optimized reaction buffers. Solulink recommends Diafiltration spin columns (S-4004) for desalting amino-oligonucleotides greater than 5,000 daltons.

Oligonucleotide Buffer Exchange & Desalting Procedure

A. Oligonucleotide Sample Loading

- Oligonucleotide Preparation:
 - For oligonucleotides in solid form:
 - Spin down the vial containing the amino-oligonucleotide (30-100 OD₂₆₀ units) to ensure all of the oligonucleotide is settled at the bottom.
 - Dissolve the oligonucleotide in 250 μ L of 1x Modification Buffer, pH 7.4.
 - For oligonucleotides in solution:
 - Vivaspin 500 dilafiltration spin filters are made to contain and process volumes of 500 μ L or less. If volumes greater than 500 μ L are to be processed, then multiple filters or loadings may be required.
- Determine the amount of OD₂₆₀ present (optional):
 - Fill two microcentrifuge tubes with 998 μ L of H₂O. Add 2 μ L of 1x Modification Buffer, pH 7.4 to the first tube, which will be used as the "Blank". Add 2 μ L of the oligonucleotide solution to the second tube.
 - At 260nm, blank the spectrophotometer using the "Blank" tube, then take the reading for the second tube. Divide that number in half to obtain the OD₂₆₀/ μ L.
 - Using the OD₂₆₀/ μ L and the known total volume, determine the amount of starting OD₂₆₀.
- Add 250 μ L of appropriate buffer to the 248 μ L oligonucleotide solution.
- Add the total solution (498 μ L) to the diafiltration concentrator body and place into the centrifuge with an appropriate balance. Make sure the concentrator body lid is closed.

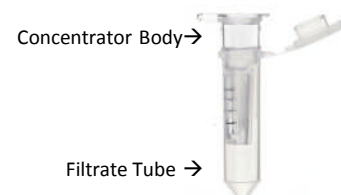


Figure 1: Diafiltration Spin Column

B. Oligonucleotide Desalting and Buffer Exchange

- Centrifuge for 7-10 minutes at 15,000 x g.
- After the spin, check to make sure the volume is at 50 μ L or less in the concentrator body. If the volume is greater than 50 μ L, spin the column longer until the volume is \leq 50 μ L.
- The excess buffer will flow into the collection tube. Collect the flow through after each spin in a separate microcentrifuge tube. This is a precautionary step to ensure no oligonucleotide is lost if the membrane breaks.
- Add 450 μ L of desired buffer to the concentrator body and pipette up and down to mix the solution without touching the membrane. Make sure not to touch or damage the filter surface with the pipette tip.
- Repeat steps 5 to 8 an additional four times.
- After the last spin, transfer the desalted oligonucleotide to a new microcentrifuge tube.
- Determine the amount of OD₂₆₀ present and OD₂₆₀/ μ L. Refer to step 2 for details. The volume of the oligonucleotide solution can be adjusted by adding more buffer until the OD₂₆₀/ μ L solution is between 0.2 to 0.5 OD₂₆₀/ μ L.