

Protocol for Modification of Amino-Oligonucleotides

Materials Required

1X Modification Buffer (0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.4)
1X Modification Buffer (0.1 M sodium phosphate, 0.15 M sodium chloride, pH 6.0)
VIVASPIN 500 Spin Filter Unit (Cat. # VS0112, Sartorius)
Microcentrifuge (e.g. Eppendorf, IEC Micromax or similar)
P-2, P-200, and P-1000 micropipettes
UV-spectrophotometer



Figure 1. VIVASPIN 500 Filter Unit

Modification Protocol

1. Desalt the amino-oligonucleotide into 1X Modification buffer (pH 7.4) (refer to PDF protocol 'Desalting Amino-Modified Oligonucleotides').
2. After desalting, measure the concentration of the amino-oligo using a spectrophotometer. Record the measured concentration in OD₂₆₀/ul.
3. Use the calculator to input oligo information including the name, molar extinction coefficient, oligo concentration (OD₂₆₀/ul) and the total volume of amino-oligo available (ul) for modification.
4. Using the calculator also input linker information including the name and molecular weight of the linker being used to modify the oligo (select from drop down menu in calculator) along with the mass of linker weighed and the volume (ul) of solvent (DMF or DMSO) used to dissolve the linker.
5. Mix the indicated volumes of amino-oligo, organic solvent (DMF or DMSO), and linker to initiate the modification reaction.
6. Incubate the reaction for 2 hours at room temperature.
7. After the reaction is complete, spin the reaction tube at 15,000 x g in a microfuge for 15 minutes to collect any insoluble salts that form during the reaction.
8. Carefully transfer the clear supernatant (without disrupting any visible pellet) to a new VIVASPIN 500 spin filter unit and adjust the volume to 500 ul in the concentrator body by addition of 1X Modification buffer (pH 6.0)
9. Desalt the reaction mixture (refer to PDF protocol 'Desalting Modified-Oligonucleotides') into 1X Modification Buffer (pH 6.0). After desalting, adjust the final modified oligo concentration (i.e. OD₂₆₀/ul) to between 0.3 and 0.5 OD₂₆₀/ul by addition of 1X Modification buffer (pH 6.0) before conjugating the modified oligo to a protein.