

Oligonucleotide Concentration Determination

Determination of the concentration of an oligonucleotide (in units of OD₂₆₀/μl) is required to verify the amount of material present in OD₂₆₀ units. The concentration and amount of oligo is used in modification and conjugation procedures to ensure the optimal stoichiometry is used in these reactions. Follow the instructions below for oligonucleotide preparation, then determine the oligo concentration using either a conventional UV-Vis spectrophotometer and 1-cm pathlength quartz cuvette or a NanoDrop™ spectrophotometer.

Materials Required:

Reagents	Equipment
Ultrapure Water	1.5 ml Microcentrifuge Tubes
Oligo Buffer	UV-Vis Spectrophotometer or NanoDrop Spectrophotometer

Oligonucleotide Preparation (For Protocols Below)

- For oligonucleotides in solid form:
 - Spin down the vial containing lyophilized oligonucleotide to ensure all material is settled at the bottom.
 - Dissolve the oligonucleotide in 1X Modification Buffer, pH 8.0, or another suitable buffer at approximately 0.5 OD₂₆₀/μl.

Example: If the vial contains 35 OD₂₆₀ units of oligonucleotide, use 70 μl of buffer (35 OD₂₆₀/70 μl = 0.5 OD₂₆₀/μl)
- For oligonucleotides already in solution:
 - Ensure the buffer solution does not interfere significantly with the 260 nm absorbance.

Protocols:

Conventional Spectrophotometer Concentration Determination

- Add 998 μl of water to two microcentrifuge tubes.
- Add 2.0 μl of buffer to the first tube, if applicable, which will be used as a Blank.

Note: The buffer should be similar to the buffer that the oligonucleotide is dissolved in (e.g., Modification Buffer, pH 8.0).
- Add 2.0 μl of the stock oligonucleotide solution to the second tube.
- Vortex both tubes to mix.
- Using a UV-transparent plastic or quartz cuvette, blank the spectrophotometer at 260 nm using the blank solution.
- Read the 260 nm absorbance of the diluted oligo solution.
- Divide the absorbance value by 2 to calculate the OD₂₆₀/μl concentration of the stock oligonucleotide.
- Using the OD₂₆₀/μl concentration and the stock oligonucleotide volume, calculate the amount of oligo available by multiplying the concentration in OD₂₆₀/μl by the volume in μl.

NanoDrop Concentration Determination

- Add 198 μl of water to two microcentrifuge tubes.
- Add 2.0 μl of buffer to the first tube, if applicable, which will be used as a Blank.

Note: The buffer should be similar to the buffer that the oligonucleotide is dissolved in (e.g., Modification Buffer, pH 8.0).
- Add 2.0 μl of the stock oligonucleotide solution to the second tube.
- Vortex both tubes to mix.
- Select the "Nucleic Acid" menu option on the NanoDrop and initialize the instrument using water, if required (NanoDrop 1000 only).
- Blank the instrument at 260 nm with 2 μl of the blank solution.
- Measure the 260 nm absorbance of the diluted oligo solution as displayed in the 10-mm (1-cm) pathlength window. Record the A₂₆₀ value.
- Divide this number by 10 to calculate the OD₂₆₀/μl concentration of the stock oligonucleotide.
- Using the OD₂₆₀/μl concentration and the stock oligonucleotide volume, calculate the amount of oligo available by multiplying the concentration in OD₂₆₀/μl by the volume in μl.