

Oligonucleotide Concentration Determination Protocol



Materials Required

Reagents	Catalog #	Equipment
Nuclease-free H ₂ O	n/a	1.5 mL Microcentrifuge Tubes
Desired Buffer	n/a	UV-Spectrophotometer

Proper determination of the concentration of oligonucleotide (OD₂₆₀/μL) is essential to verify amount of OD₂₆₀ units present. The concentration and amount of OD₂₆₀ units is used in modification and conjugation reactions.

Oligonucleotide Concentration Determination

A. 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 μL of 1x Modification Buffer, pH 7.4.
- For oligonucleotides already in solution:
 - Check that buffer solution does not interfere with A₂₆₀ absorbance.

B. A₂₆₀ Absorbance Reading / Determination

- Fill two microcentrifuge tubes with 998 μL of H₂O.
- Add 2 μL of buffer to the first tube, which will be used as the "Blank".
 - Buffer should be similar to the buffer that the oligonucleotide is dissolved in (e.g. Modification Buffer, pH 7.4).
- Add 2 μL 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/μL.
- Using the OD₂₆₀/μL and the known total volume, determine the amount of starting OD₂₆₀.