

# Oligonucleotide Concentration Determination Protocol



## Materials Required

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

Proper determination of the concentration of oligonucleotide (OD<sub>260</sub>/μL) is essential to verify amount of OD<sub>260</sub> units present. The concentration and amount of OD<sub>260</sub> 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 OD<sub>260</sub> 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 A<sub>260</sub> absorbance.

### B. A260 Absorbance Reading / Determination

- Fill two microcentrifuge tubes with 998 μL of H<sub>2</sub>O.
- 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 OD<sub>260</sub>/μL.
- Using the OD<sub>260</sub>/μL and the known total volume, determine the amount of starting OD<sub>260</sub>.