DNA/RNA Quantification

A260/A280 Purity Ratios

Pure DNA: ~1.8 | Pure RNA: ~2.0 | Protein contamination: <1.8

Absorbance (NanoDrop)

Fast, 1-2 µL. A260/280, A260/230 ratios. Contamination detection.

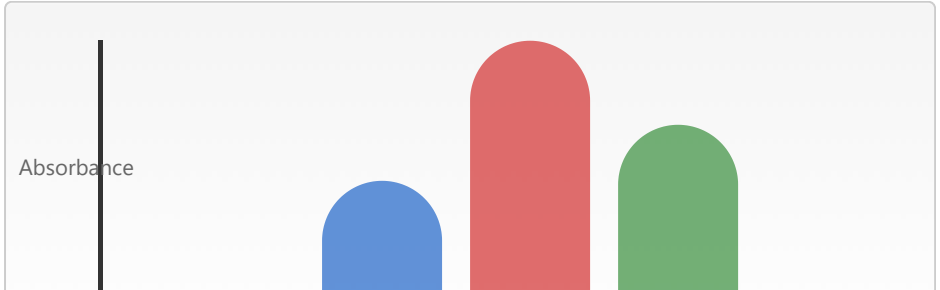
Fluorometric (Qubit)

Selective dyes. More accurate, less contamination sensitivity.

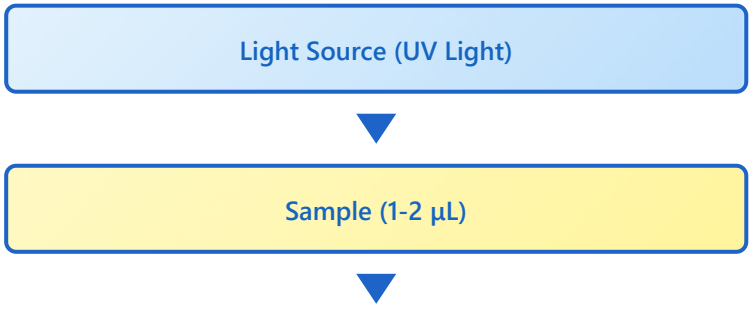
📊 Detailed Explanation and Principles

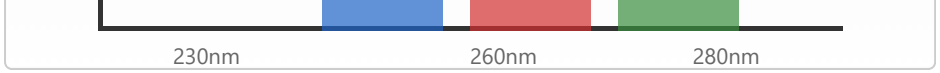
1. Absorbance Method (NanoDrop) - Spectrophotometry

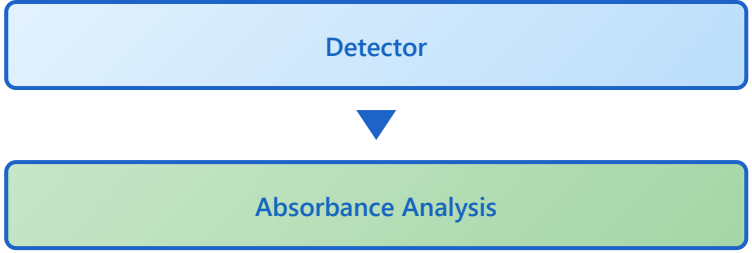
Absorption Spectrum



NanoDrop Principle





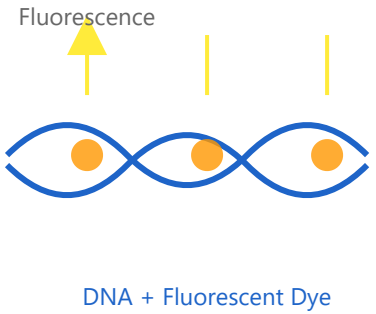


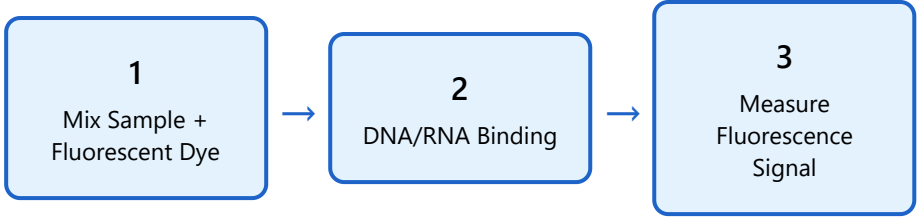
- **Measurement Principle:** Utilizes the property of nucleic acids absorbing UV light at 260nm wavelength
- **A260/A280 Ratio:**
  - Pure DNA: ~1.8 (no protein contamination)
  - Pure RNA: ~2.0 (RNA has higher ratio)
  - <1.8: Protein contamination suspected (proteins absorb at 280nm)
- **A260/A230 Ratio:** Ideal range 2.0-2.2 (low values indicate salt, phenol contamination)
- **Advantages:** Fast, minimal sample volume (1-2 μL), simple operation
- **Disadvantages:** Susceptible to contaminants, relatively lower accuracy

2. Fluorometric Method (Qubit) - Fluorescence-based Quantification

Fluorescence Principle

Measurement Process





- **Measurement Principle:** Uses fluorescent dyes that specifically bind to DNA/RNA
- **Selective Dyes:**
  - dsDNA-specific dye: Measures only double-stranded DNA
  - ssDNA dye: Measures single-stranded DNA
  - RNA-specific dye: Selectively measures RNA only
- **Advantages:** High accuracy, minimal contamination interference, high specificity
- **Disadvantages:** Relatively higher cost, requires dye reagents
- **Applications:** NGS, PCR, and experiments requiring precise quantification

3. Comparison of Two Methods

Comparison	NanoDrop (Absorbance)	Qubit (Fluorescence)
Measurement Principle	UV absorbance (260nm)	Fluorescent dye binding
Sample Volume	1-2 µL (minimal)	1-20 µL

Comparison	NanoDrop (Absorbance)	Qubit (Fluorescence)
Measurement Time	Very fast (seconds)	Fast (minutes)
Accuracy	Moderate (contamination-sensitive)	High
Purity Assessment	Possible (260/280, 260/230)	Not possible
Selectivity	Low (all nucleic acids)	High (specific nucleic acids)
Contamination Sensitivity	High (protein, salt effects)	Low
Cost	Low (no reagents needed)	High (dye reagents)
Primary Use	Quick screening, purity check	Precise quantification, NGS prep

4. Experimental Tips and Precautions

✅ NanoDrop Usage

- Calibrate with blank before measurement
- Ensure no bubbles on sample surface
- Clean thoroughly after measurement
- Always check 260/280 ratio

✅ Qubit Usage

- Always prepare standard curve
- Select appropriate dye
- Store in dark conditions
- Measure after fluorescence stabilization