Application Note

dsDNA Quantification by UV Absorbance
For Automated NGS Sample Preparation

Accurate quantification and quality assessment of nucleic acids are essential steps in next-generation sequencing (NGS) workflows. Following extraction, DNA samples must be evaluated for concentration, purity and suitability for downstream processing to ensure successful library preparation and reliable sequencing performance. UV absorbance remains the preferred method for post-extraction DNA quantification due to its speed, simplicity, label-free operation, and ability to retain precious sample. Integrating UV absorbance measurements is a key step toward end-to-end automation in sample preparation workflows.

This application note introduces an automated on-deck UV absorbance workflow using the eviDense UV Photometer and presents performance data demonstrating accuracy and precision in comparison with a conventional benchtop spectrophotometer.

NGS Sample Preparation and Quality Control

NGS workflows consist of a series of interconnected steps in which nucleic acid samples are transformed into sequencing-ready DNA libraries. Each stage – from extraction to library preparation – relies on accurate and consistent quantification and quality assessment of the input material.

Quality control (QC) is performed at several points throughout the workflow to verify the integrity and suitability of DNA samples for downstream processing. Commonly used techniques include qPCR, UV absorbance, and fluorescence-based methods.

Verification of DNA concentration and purity immediately after extraction is a critical step in NGS sample preparation. At this stage, UV absorbance provides a practical and widely adopted solution for rapid, label-free DNA quantification with full sample recovery. Accurate QC at post-extraction stage ensures:

- Sufficient DNA is available for downstream applications
- Samples below minimum input requirements are identified early
- Correct normalization is performed prior to fragmentation
- Contamination due to proteins, phenol, or salts is detected early

Together, these checks ensure adequate yield, required purity and overall sample integrity – factors that directly influence downstream library prep and sequencing performance.

Principle of UV Absorbance Method for dsDNA Quantification

The UV absorbance method exploits the intrinsic optical properties of nucleic acids. DNA and RNA exhibit a strong absorbance maximum at 260 nm, a wavelength at which the aromatic bases efficiently absorb ultraviolet light. In accordance with the Beer-Lambert law, the absorbance measured at 260 nm is directly proportional to the concentration of nucleic acid in the solution, allowing quantitative determination without the need for dyes or labels.

To improve accuracy, measurements are typically accompanied by a background correction at 340 nm,

Figure 1: NGS sample preparation workflow

a wavelength at which nucleic acids show negligible absorbance. Subtracting this background signal compensates for instrument noise, light scattering, and potential baseline shifts, thereby enhancing the precision of the concentration measurement.

In addition to determining concentration, UV absorbance enables rapid assessment of sample purity using characteristic absorbance ratios:

- A260/A280 provides information on protein contamination, as proteins absorb strongly at 280 nm. Pure dsDNA typically yields a ratio of ~1.8.
- A260/A230 helps identify contaminants such as phenol and salt, which absorb at 230 nm. High-quality DNA usually exhibits ratios of approximately 2.0 – 2.2.

Together, these measurements enable reliable determination of DNA concentration and assessment of sample purity prior to downstream processing.

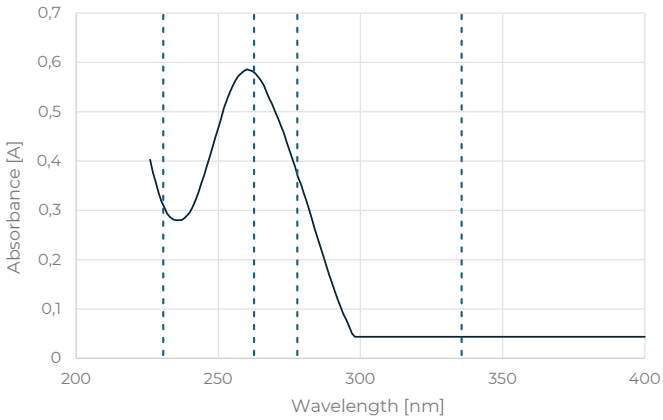

Figure 2: Example UV Absorbance Spectrum of dsDNA

Manual Versus Automated Workflow

Traditional DNA quantification is typically performed using either benchtop spectrophotometers or microplate readers. While widely used, these methods introduce several challenges:

- **Manual pipetting:** increases hands-on time and introduces operator-induced errors
- **Manual data transfer:** adds workload and risk of transcription errors
- **Limited integration into automated workflows:** requires dedicated bench space and creates bottlenecks in medium-high-throughput applications
- **Pathlength and surface-related variability in microplate readers:** reduces measurement consistency and accuracy

To address these limitations, the eviDense UV Photometer provides an automated, on-deck, cuvette-based UV absorbance workflow that integrates seamlessly with liquid handling systems. Measuring at four wavelengths – 260, 230, 280 and 340 nm – this on-deck module supports accurate dsDNA quantification and purity assessment.

Figure 3: eviDense UV Photometer

Integrating UV absorbance measurement directly into the liquid handling workflow offers the following key advantages:

- **Precision and reproducibility:** Automation removes manual pipetting variability and ensures consistent sample handling
- **Walk-away processing:** once samples are loaded on the liquid handler, the process runs fully hands-free for up to 192 samples
- **Sample retention:** Samples can be fully recovered and used for downstream processing
- **Data integrity:** Automated digital data capture provides traceability, reduces errors and supports downstream analytics
- **Space saving:** A single SBS deck position is required

Performance Data

The following study demonstrates UV absorbance measurements performed on a liquid handling system and compared with a benchtop spectrophotometer. The automated workflow includes tip pick up, sample aspiration, cuvette loading, transfer to the eviDense UV detector unit, multi-wavelength measurement, and

cuvette ejection with the sample aspirated back into the tip for optional downstream processing.

Figure 4 shows measurements conducted with salmon sperm dsDNA in Tris-EDTA (TE) buffer. Eight replicates of each sample were measured on the eviDense UV Photometer, integrated into a liquid handler, and on a standalone benchtop spectrophotometer ("reference spectrophotometer").

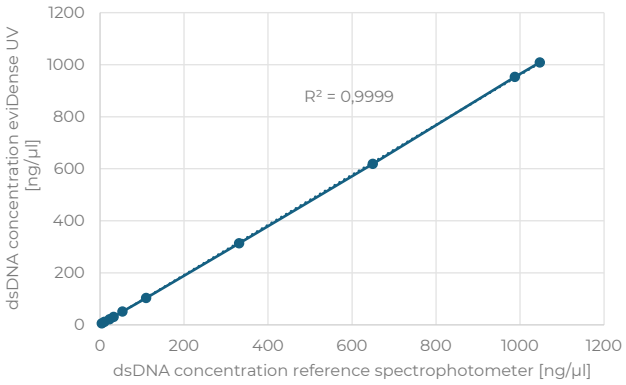

Figure 4: Correlation of dsDNA concentration measurements of salmon sperm DNA: eviDense UV Photometer versus reference UV-Vis Spectrophotometer

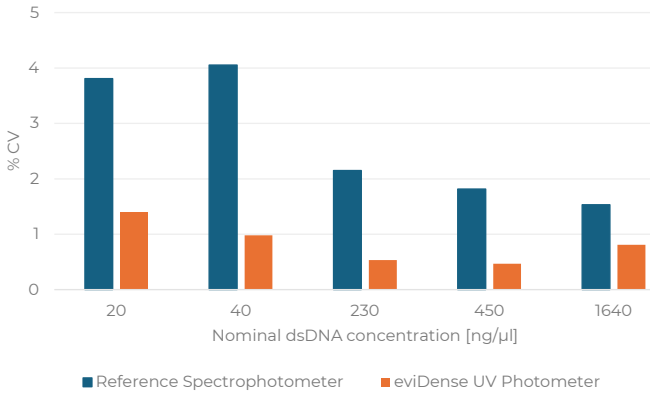

Figure 5: Coefficient of variation (CV) of dsDNA concentration measurements in 8 replicates of salmon sperm DNA: eviDense UV Photometer versus reference UV-Vis Spectrophotometer

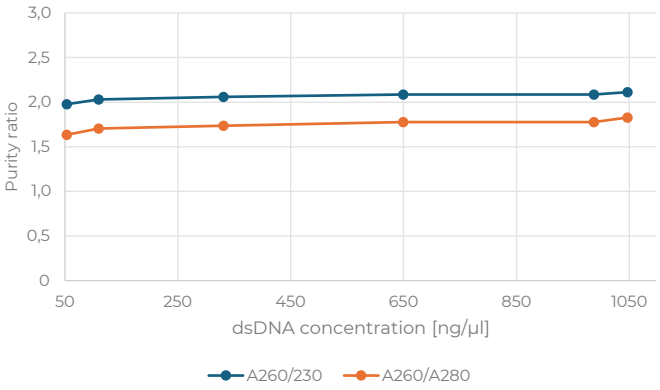

Figure 6: Purity ratios measured at different dsDNA concentrations

Measurements obtained with eviDense UV showed excellent agreement with the reference UV-Vis Spectrophotometer (R^2=0.9999). In addition, the eviDense UV demonstrated consistently low coefficients of variation (CV), indicating a high measurement precision.

Figure 6 summarizes the nucleic acid purity ratios A260/A280 and A260/A230. The A260/A280 ratio is commonly used to assess protein contamination, while the A260/A230 ratio provides an indicator of contamination from salts and phenol. Across all samples, the measured purity ratios were close to the expected values for high-quality DNA, approximately 1.8 for A260/A280 and 2.0 – 2.2 for A260/A230.

Summary and Conclusion

UV absorbance remains a powerful and efficient method for post-extraction DNA quantification and purity assessment in NGS workflows. By integrating the eviDense UV Photometer directly onto the liquid handling platform, the automated workflow eliminates manual pipetting steps, reduces transcription errors, and ensures consistent optical conditions across large sample batches.

Performance data obtained using salmon sperm dsDNA demonstrate that the automated workflow delivers high accuracy and precision comparable to a benchtop spectrophotometer, while providing the advantages of walk-away processing, sample retention, and complete digital traceability. These results establish automated on-deck UV absorbance measurement as a robust and scalable approach for early QC in NGS sample preparation – ultimately contributing to more efficient and reliable sequencing workflows.

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For more information, consult
www.on-deck-photometer.com