DeNovix®

Absorbance and Fluorescence Quantification

An Application Guide to Sample QC

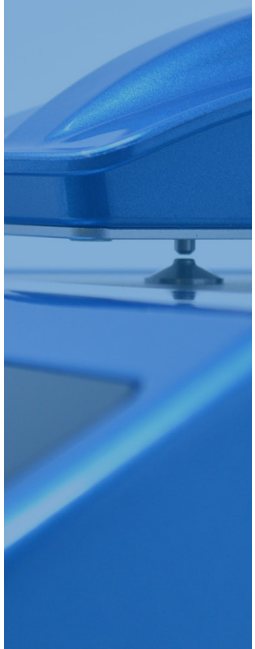
30-JAN-2023

About this eBook

Fluorescence and UV-Vis Absorbance are complementary measurement techniques for the quantification and quality control of samples. Each method has advantages depending on the application and sample being quantified.

In this eBook, you'll find resources that discuss the advantages of each method and when to employ Absorbance, Fluorescence or both.

Contents



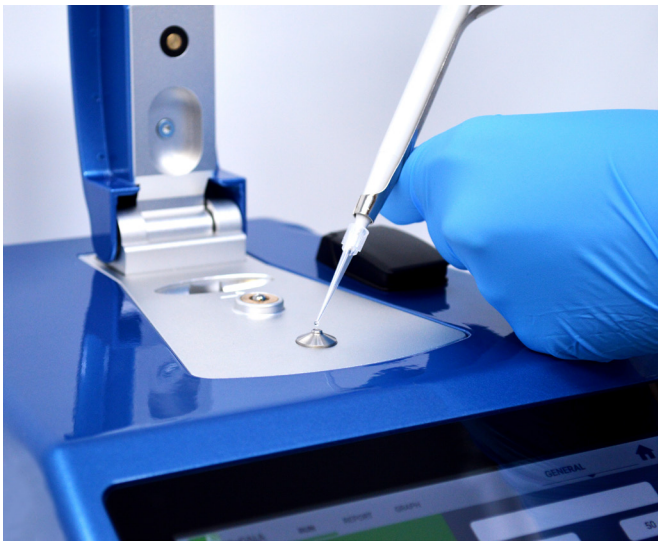
Absorbance and Fluorescence Quantification

The DeNovix™ DS-11 Series Spectrophotometer / Fluorometer enables precise absorbance & fluorescence quantification across a wide dynamic range. The dual mode spectrophotometer is equipped with SmartPath™ technology, which facilitates accurate and reproducible measurements for both cuvette and 1 µL absorbance modes. The proprietary optical core of the fluorescence component utilizes LED excitation sources and highly sensitive photodiodes capable of detecting minute amounts of fluorescence across four wavelength ranges.

Basics of Absorbance Measurements

UV-Vis absorbance measurements have long been a standard method for quantification of purified biomolecules in the life science laboratory. This method allows for the rapid detection of molecules based on their absorbance profiles at specific wavelengths.

Absorbance also provides an indication of sample contamination. The shape of the absorbance spectrum will change based on the presence of other molecules that absorb at or near the same wavelengths as the molecule of interest.



Basics of Fluorescence Measurements

Fluorophores are molecules that absorb light at one wavelength (excitation wavelength) and emit light at another (emission wavelength). Certain fluorophores' structures can be manipulated to fluoresce only when bound to a specific molecule (e.g., double-stranded DNA). Fluorescence assays use this binding specificity to establish a direct correlation between the amount of fluorescence emitted by a sample and the concentration of the biomolecule of interest in solution.

By mixing a fluorophore with a sample of known concentration and measuring the Relative Fluorescent Units (RFU), a relationship between concentration and measured RFU can be plotted and used as a standard curve. The emission of the same fluorophore, bound to unknown samples, can then be plotted against this standard curve to determine the sample concentration.

Advantages of Absorbance and Fluorescence Methods

Absorbance

- Reagents are not required. The measured absorbance is a direct result of the molecule of interest absorbing light at a known wavelength.
- The amount of light absorbed corresponds directly to the concentration of the molecule of interest.

Fluorescence

- High Sensitivity: Due to the high extinction coefficient of the fluorophore, fluorescence assays are extremely sensitive, allowing for the detection of molecules at concentrations hundreds of times lower than what is detectable by traditional absorbance.
- Specificity: The binding properties of the fluorophore make these methods highly selective for specific molecules. These assays are ideal for samples that may contain contaminants that would interfere with an absorbance measurement.

Comparing Absorbance and Fluorescence Results

When comparing results from absorbance-based methods to fluorescence-based methods, it is important to consider the specificity of each method. Absorbance measurements at 260 nm, for example, are not selective for dsDNA since ssDNA and RNA also absorb at 260 nm. Any absorbance measurement at 260 nm will be a measure of all nucleic acids in a sample and any contaminants that are present, including proteins. This is true for all types of measurements, not just nucleic acids. Absorbance methods are well-suited to measuring pure samples for this reason.

In contrast, fluorescence assays are highly specific for a given analyte, including but not limited to dsDNA, ssDNA, RNA or proteins. Generally, the concentration of a sample measured by absorbance is greater than the concentration measured by fluorescence methods.

Summary

Absorbance and fluorescence are distinct but complementary methods for quantification and quality assessment of samples. Quantitation via absorbance using the microvolume or cuvette based capabilities of the DS-11 Series is ideal for the rapid and accurate measurement of purified samples, including nucleic acids and proteins. Absorbance will also give information on potential contamination.

Fluorescence quantitation, using a secondary reporter fluorophore, is ideal for samples that fall below the detectable threshold for UV-Vis absorbance. In some cases, fluorescence quantitation methods can also be used to detect samples in the presence of contaminants or buffer elements that would interfere with UV-Vis measurements.

> **The DS-11 Series Spectrophotometer / Fluorometer integrates UV-Vis and fluorescence capabilities in a small bench top footprint.**

The DS-11 Series Spectrophotometer / Fluorometer integrates UV-Vis and fluorescence capabilities in a small bench top footprint. DeNovix offers several instrument models with combinations of microvolume absorbance, cuvette absorbance and fluorescence measurement modes. Both absorbance and fluorescence methods share the same EasyApps™ software and sample export features, making data analysis fast, easy and intuitive.

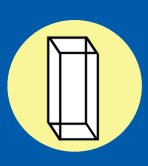
Sample QC: Absorbance or Fluorescence?

Absorbance

Absorbance methods directly measure the amount of light absorbed by a sample at a specific wavelength. The absorbance of light is proportional to concentration of the sample allowing quantification.

Microvolume

- *1 µL Sample Volume*
- *No Sample Dilutions Required*



Cuvette

- *Larger Sample Volume*
- *Higher Sensitivity than Microvolume*

Fluorescence

Fluorophores are bound to the sample of interest and excited with a specific wavelength of light. Emission is measured at a higher wavelength. Sample fluorescence is compared to known standards.

0.5ml PCR Tubes

- *200 µL assay volume*
- *1 to 20 µL of sample*
- *Choose from a wide range of fluorophores and assay kits*

Applications

Absorbance Apps

dsDNA *RNA* *ssDNA* *Protein A280* *Peptides*

Kinetics *Labeled DNA / Protein* *OD600* *Colori-metrics* *UV/Vis*

Fluorescence Apps

dsDNA ***RNA*** ***ssDNA***

Protein ***Basic Fluorometer*** ***Custom Assays***

Feature Comparison

0.75 ng/µL	Sensitivity	0.0005 ng/µL
Not analyte specific	Specificity	Highly specific
37500 ng/µL	Dynamic Range	4000 ng/µL
0.5 to 1 µL	Sample Volume	1 to 20 µL
Detects co-extracted contaminants	Detect Contamination	No contamination information
Zero cost/sample	Assay Cost	Range of assays
Load and measure in seconds	Speed	Assay set up required

The Verdict

Quick and easy method to quantify a wide range of samples without any assay cost or set up time. Microvolume measurements are possible over a wide dynamic range covering all commonly used samples concentrations. Contaminants can be detected allowing additional quality control.

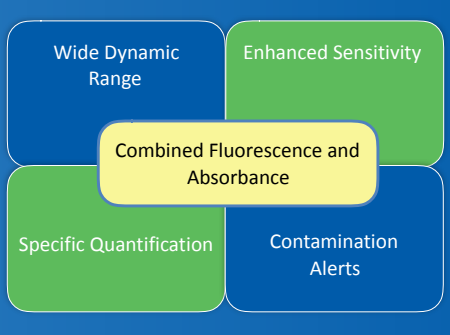
Fluorescence methods benefit from enhanced sensitivity allowing quantification to sub-picogram per micro liter concentrations. Assays are specific for the analyte under investigation leading to highly specific quantification, without interference from contaminants. Assay kits and known-concentration standards are required.

Combining Measurements

DS-11 FX Series Spectrophotometers / Fluorometers

- The most sensitive 1µL UV-Vis Spectrophotometer
- Cuvette Absorbance with heater
- Integrated, best-in-class Fluorometer

Total Sample QC



The DS-11 FX Series enables both absorbance and fluorescence measurements in one instrument. The combination of absorbance and fluorescence provides greater confidence and accuracy when measuring samples and is a protocol requirement in applications such as Next Generation Sequencing.

Want to learn more or try the DS-11 in your lab?

FREE TRIAL

DeNovix®

www.denovix.com

Purity Ratios Explained

It is common practice for molecular biologists to use the ratio of the measured spectrophotometric absorbance of a sample at 260 nm compared to the value measured at 280 nm as an assessment of purity for nucleic acid and, to a lesser extent, protein samples. Many researchers also look at the ratios of the 230 nm and 260 nm absorbance values as an important secondary measure of purity before using samples in time-consuming or expensive downstream applications.

An example of a nucleic acid sample and a protein sample as measured on a DeNovix DS-11 Spectrophotometer are shown in Figures 1 and 2 below:

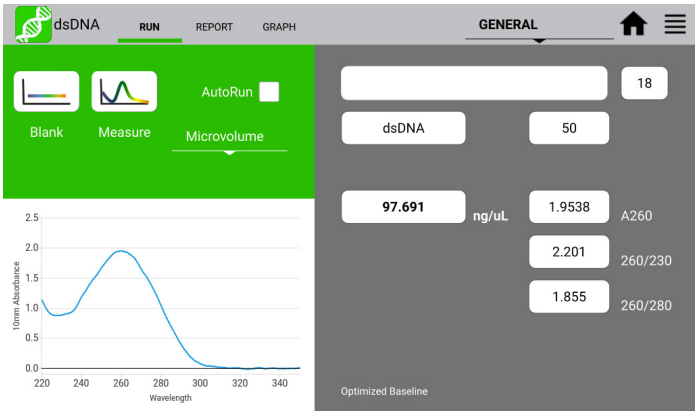


Figure 1. Typical dsDNA spectrum.

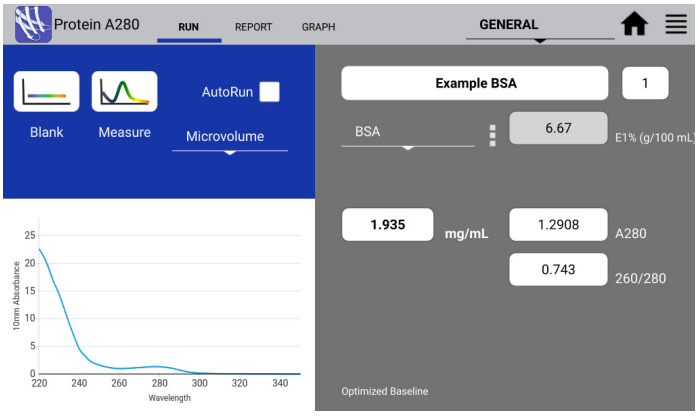


Figure 2. Typical BSA spectrum.

Compare the DeNovix DS-11 Series to the Thermo Fisher NanoDrop™ One Spectrophotometer

260/280 Nucleic Acid Purity Ratios

260/280 ratios are routinely used to determine the purity of nucleic acid measurements. This ratio is most commonly used to determine the presence of protein and or phenol in the isolated nucleic acid sample.

> **260/280 ratios are routinely used to determine the purity of nucleic acid measurements.**

Table 1 describes a general acceptable range for these ratios; however, they are not a guarantee of sample purity.

Table 1. Acceptable Range for 260/280 Ratios

Sample Type	Ideal	High	Low
DNA	~1.8	>2.0	<1.7
RNA	~2.0	>2.2	<1.9
Possible contamination		Basic	Acidic Phenol or Protein

260/230 Nucleic Acid Purity Ratios

The 260/230 ratio is used to indicate the presence of unwanted organic compounds such as Trizol, phenol, Guanidine HCL and guanidine thiocyanate.

Generally acceptable 260/230 ratios are in the range of 2.0 – 2.2. Values higher than this may indicate contamination with the aforementioned compounds.

Common Nucleic Acid Contaminants

Despite the best efforts of the researcher, residual contaminants often remain in solution with nucleic acids following chemical isolation. The presence of these can lead to an incorrectly high concentration reading or the disruption of downstream processes.

Protein 260/280 Purity Ratios

DNA is a common contaminant of proteins isolated from whole cell lysates. When measuring purified proteins, the 260/280 ratio can be a useful tool to determine the purity of an isolated protein. An ideal 260/280 ratio for common proteins is 0.6. Higher ratios may indicate the contamination of isolated proteins with DNA.

Alternatively, the buffer used to isolate the sample protein may include components that absorb strongly in the UV region. Buffers and buffer components that fit this description include RIPA buffers, Triton, DTT, urea and thiourea.

Note: Colorimetric spectrophotometric methods such as the BCA or Bradford assays are generally recommended when working with buffers with UV region absorbance.

Troubleshooting Purity Ratios

The three most common causes of abnormal 260/280 ratios are listed below:

Common Causes of Abnormal 260/280 Ratios

- **Sample contamination** by residual phenol, guanidine and other reagents used in the isolation protocol. Carbohydrates are a common contaminant associated with nucleic acid isolations from plant sources.
- **Samples are very dilute** and concentrations are close to the lower detection limit. Inaccurate ratios may be observed for nucleic acids samples with concentrations <10 ng/µL.
- **An inappropriate solution was used for the Blank measurement.** The Blank solution should be of a similar ionic strength and pH as the sample solution. Using water for the Blank measurement for samples dissolved in TE may result in low 260/230 ratios.

Microvolume vs. Cuvette

It has been reported that small changes in the pH of a solution may result in variances in nucleic acid 260/280 ratios[1]. Acidic solutions may under-represent the 260/280 ratio value by 0.2 – 0.3, while a basic solution may over-represent the ratio value by 0.2 – 0.3. If comparing ratios for samples measured on the microvolume mode and then diluted to be measured with a cuvette, ensure that the undiluted and diluted sample are at the same ionic strength and pH.

Variance from Other Instrument Brands

It is not unusual to observe slight differences in purity ratios when measuring the same sample on different spectrophotometers. Up to about a 0.4 difference in the 260/280 ratio may be observed when measuring the same nucleic acid sample on two spectrophotometers that are working within a 1 nm wavelength accuracy specification.

The absorbance of nucleic acid at 260 nm is measured within a plateau region of the spectrum, while the 280 nm absorbance is generally measured on a steep sloped portion of the spectral curve. The measurement of one value on a plateau and another on a slope means that a slight shift in wavelength accuracy will have a large effect on 260/280 ratios.

The DS-11 Series is specified to a wavelength accuracy of 0.5 nm. Instruments with larger wavelength accuracy specifications are less able to accurately report 260/280 ratios.

Summary

The ratio limits presented in this note are generally accepted ideal values. It is important to empirically determine the ratio limits that predict sample functionality in your downstream applications.

The DS-11 Series EasyApps software provides automatic alerts for samples outside these standard values, or custom thresholds can be entered.

References

1. William W. Wilfinger, Karol Mackey, and Piotr Chomczynski, Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity: BioTechniques 22:474-481 (March 1997).

dsDNA Quantification for NGS Quality Control

ANDREW JONES | MARKET DEVELOPMENT MANAGER | DENOVIX INC.

In this interview, Andrew Jones discusses the importance of quality control in next-generation sequencing and the effect that a poorly followed protocol can have on experimental results.

How important is quality control of dsDNA samples for next generation sequencing? How can artifacts affect the quality of subsequent data analyses?

Quantity and quality of input samples are two key considerations in successful library preparation for next generation sequencing. The ultimate success or failure of a library preparation is often strongly linked to using an accurately quantified amount of input DNA.

Most next-gen sequencer providers recommend the quantification of input sample by analyte-specific fluorometric quantification, for example the DeNovix dsDNA fluorescence quantification assays as well as Thermo Fisher PicoGreen™ and Thermo Fisher Qubit™ (Qubit™) assays. These methods utilize intercalating dyes specific for dsDNA and do not bind to other nucleic acid species that may be present.

In addition to quantification, it is also important that samples are free from potential enzymatic inhibitors. To assess the purity of a sample, microvolume UV-Vis spectrophotometry is recommended.

Image credit: Zita | Shutterstock

What are the advantages of being able to conduct absorbance and fluorescence measurements with one system?

The ability to combine both absorbance and fluorescence quantification methods provides a number of important advantages for researchers. For NGS labs, it enables researchers to obtain quantitative and qualitative information on a single platform for both pre- and post-PCR sample QC while using minimal amounts of sample.

Combining results from fluorescence quantification methods with purity ratio and concentration measurements gained through UV-Vis analysis enables better-informed decisions on whether your sample is likely to perform well in sequencing.

The difference between concentration measurements using absorbance and fluorescence may also give an indication of the efficiency of your extraction in isolating only the nucleic acid of interest as the absorbance value will also include absorbance of all nucleic acids (single nucleotides, oligos, RNA, dsDNA or ssDNA).

> **An instrument capable of performing absorbance and fluorescence also extends the utility of the device for life science labs without the need for multiple purchases.**

The DS-11 FX+ for example, has a four-LED fluorescence optical core that, in addition to all common nucleic acid and protein quantification assays, will also measure a wide range of other fluorophores. The DS-11 FX+ also has the option to use traditional cuvettes for UV-Vis, ideal for kinetic assays.

Want to keep reading?

Read the Full Interview

There's more to learn! Click above to read the rest of Andy's interview with News Medical Life Sciences.

DS-11 Series

SPECTROPHOTOMETER / FLUOROMETER

The DS-11 Series instruments offer intuitive full spectrum UV-Vis and fluorescence modes, making it the ideal choice for rapid nucleic acid and protein quantification.

Microvolume

Cuvette

Fluorometer

★★★★★

"Easy to use, compact and great features, easy transfer of data, customer service is excellent!"

★★★★★

"Great results, super easy to use, a must-have in every lab."

- Nucleic Acid and Protein Quantification
- Full Spectrum UV-Vis
- Integrated Fluorescence
- Widest dynamic range in the market
- Stand-alone, intuitive touchscreen interface
- Flexible Export, Wi-Fi, Email, Ethernet, & USB

Free Trial

Request a Quote

Virtual Demo

Microvolume Nucleic Acid Performance Data

The precision specification associated with a spectrophotometer is an assessment of the reproducibility of measurements. The DeNovix DS-11 Series is equipped with SmartPath T\ technology. This enables reproducible nucleic acid measurements with an absorbance precision of 0.015 AU (1 cm) or 1% (whichever is greater) at concentrations below 50 ng/µL and 1.5% or less variance at concentrations greater than 50 ng/µL dsDNA.

Linearity specifications refer to the working range of a spectrophotometer. The DS-11 has a linear range from 0.015 A – 750 A (1 cm equivalent), translating to 0.75 – 37500 ng/µL dsDNA.

To highlight the precision and accuracy of the instrument over the most commonly used range of the instrument, a dilution series of purified double stranded DNA was prepared. Five replicates of each concentration were measured using the dsDNA app.

Materials

A 30 mg/mL solution of dsDNA was gravimetrically prepared using biotechnology grade fish sperm DNA sodium salt (Amresco cat #1B1509-256) and HPLC grade water (Ricca cat #9153-1). A series of 15 dilutions ranging from ~26000 – 2 ng/µL was then prepared using the HPLC grade water.

Reference concentrations for the dilutions were determined using an Agilent 8453 (Agilent, Santa Clara CA) in a 1 mm quartz cuvette (Starna, cat #1-Q-1). The reference value for DNA solutions with absorbance values outside of the Agilent's upper range of 2.0 A (equivalent to 1000 ng/µL dsDNA) were determined by gravimetrically diluting these samples to fall within the linear range of the reference spectrophotometer.

Methods

The dsDNA app was launched and a microvolume mode Blank measurement was made using 1 µL of HPLC grade water. Five measurements were then made for each dsDNA sample concentration. Fresh 1 µL aliquots were used for each replicate measurement. The sample solution was removed between each measurement by wiping the upper and lower sample surfaces with a clean, dry laboratory wipe.

Concentrations were calculated by applying Beer's Law using 10 mm equivalent 260 nm absorbance values.

Precision Results

The reported % CVs of 1.5% or less meets the specified absorbance precision for samples > 50 ng/µL dsDNA.

Low Concentration and % Error

All spectrophotometers have a detectable level of background noise contributed by the environment and internal electronic components. The LDL is the lowest quantity of analyte that can be distinguished from this background noise. For the DS-11 microvolume mode, the LDL is 0.75 ng/µL dsDNA.

It is important to note that the % error can be high when measuring close to the LDL even for instruments meeting specifications. It is recommended that replicate measurements be made when quantifying very low concentration samples.

Table 1. DS-11 Precision Data for dsDNA

Expected ng/µL	Average ng/µL	%Error	%CV
2.00	2.80	40.0	8.29
4.05	4.88	20.5	8.82
7.38	6.83	7.45	6.65
13.49	14.21	5.34	4.18
26.74	27.66	3.44	4.28
50.23	53.31	6.13	1.35
108.26	113.0	4.38	0.35
255.23	259.9	1.83	0.60
513.26	509.2	0.79	0.35
1036.8	1020.7	1.55	0.14
2082.8	2102.1	0.93	0.17
4720.8	4628.4	1.96	0.37
10390	10118	2.62	0.67
12616	12444	1.36	0.49
26269	24295	7.50	0.79

Linearity Results

Graphical representations of the broad linear range response of the DS-11 Spectrophotometer are presented below. Target absorbances were determined as previously described.

The first graph (Figure 1) shows the linearity results for a broad concentration range. The second graph (Figure 2) shows the linearity results for just the lower concentration range to better visualize the outstanding linearity in this range.

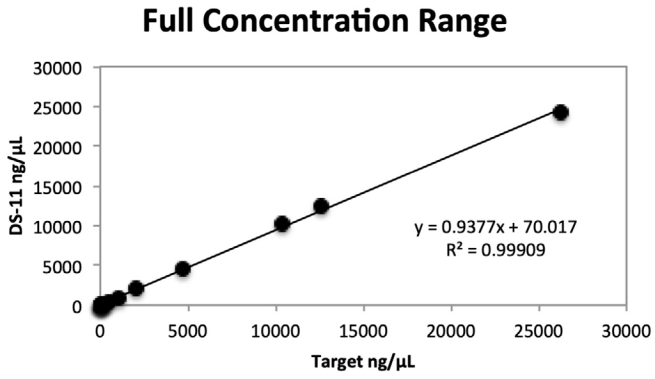


Figure 1. Full dsDNA concentration range.

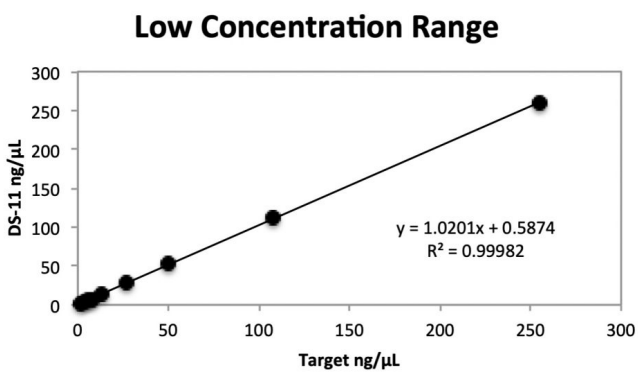


Figure 2. Low dsDNA concentration range.

Discussion

The data presented in Table 1 and graphically represented in Figures 1 and 2 demonstrate that the DS-11 Series meets the published precision and linear range specifications on nucleic acid samples commonly used in molecular biology laboratories.

SmartPath Technology automatically uses multiple pathlengths to enable absorbance measurements across a broad linear range while conserving sample. Each microvolume measurement requires only a 1 µL aliquot of sample.

The DS-11 Series microvolume mode makes it easy to measure samples with concentrations that span 4 orders of magnitude without making dilutions or using special caps or cuvettes.

The DS-11+ model, with its built-in cuvette capability, extends the lower detection range for nucleic samples down to 0.04 ng/µL. The added fluorescence capability of the DS-11 FX models in conjunction with the dsDNA fluorescence assays further extends the sensitivity down to 0.5 pg/µL.

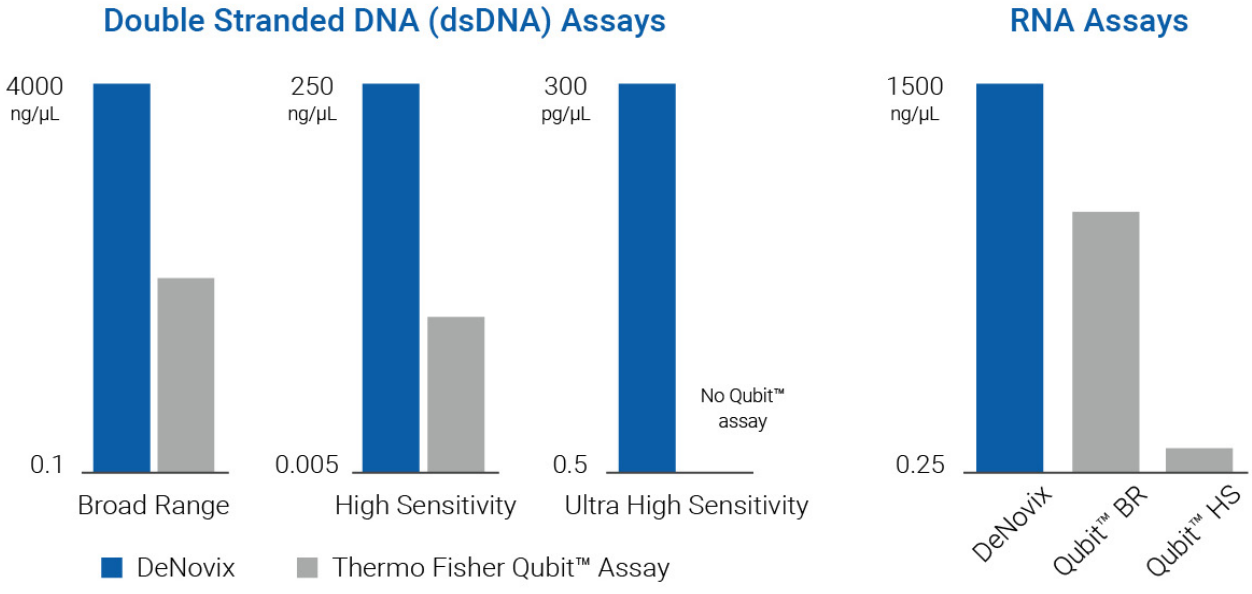
Fluorescence Quantification Assays

dsDNA AND RNA ASSAYS

DeNovix Fluorescence Assay Kits provide the greatest sensitivity and widest dynamic range available today. DeNovix offers highly specific assay kits for dsDNA and RNA.

These kits selectively and accurately quantify in the presence of common contaminants such as other nucleic acids or protein. Combine the specificity of fluorescence with the purity measurements of absorbance on the DS-11 FX or DS-11 FX+ for the optimum sample QC procedure.

Request Free Sample **Purchase Assays Now**



dsDNA Assay Concentration Limits

DeNovix Fluorescence Assays enable dsDNA quantitation over a broad range of concentrations using three distinct fluorescence assays kits.

Broad Range

Ideal for measuring sample concentrations of **0.1 – 2000 ng/µL**, with an extended upper range to **4000 ng/µL.**

High Sensitivity

Used to measure samples with concentrations ranging between **5 pg/µL – 250 ng/µL.**

Ultra High Sensitivity

This assay is optimized to enable the quantification of very low concentrations of dsDNA **0.5 – 300 pg/µL.**

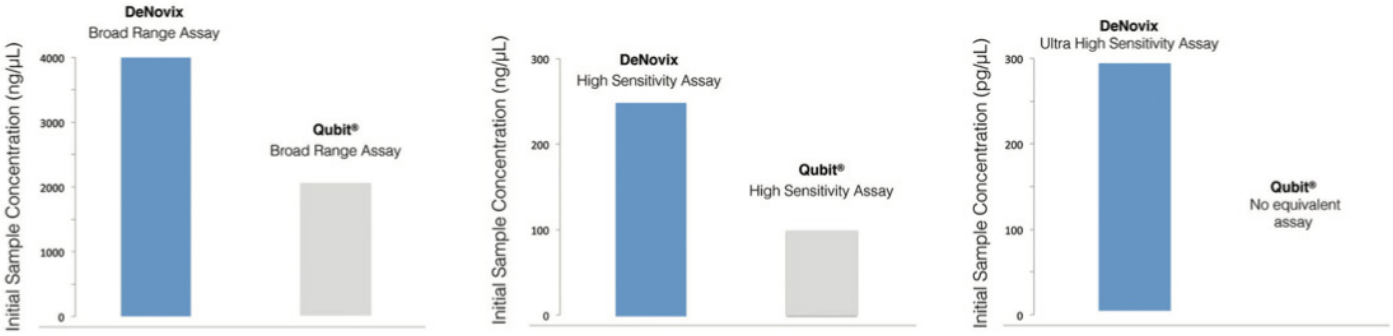


Figure 1. DeNovix dsDNA Assay Ranges. Total assay range is 0.5 pg/µL to 4000 ng/µL.

Note: When comparing reported concentration ranges for kits made by various manufacturers, it is important to review specifications based upon common nomenclature. Specifications may be reported as initial sample concentration, concentration after dilution in the assay tube or total mass of biomolecule of interest in the tube.

Concentration vs. Total Mass

Fluorescent assays are generally designed to work over the specified core assay ranges using 10 µL of sample in 190 µL of working dye reagent for a total volume of 200 µL. For some assays, the range can be extended by varying the volume ratios. The key is to stay above the minimum and below the maximum kit specific recommended total mass of dsDNA in each tube.

> **The key is to stay above the minimum and below the maximum kit specific recommended total mass of dsDNA in each tube.**

Example Minimum Kit Concentration:

Using 10 µL of the lowest initial sample concentration covered by a kit with a stated lower limit of 10 pg/µL results in a minimum absolute mass of 100 pg in 200 µL. This can also be stated as 0.5 pg/µL after dilution in the assay tube.

Note: Lower initial sample concentrations may be measured by increasing the volume (e.g., 20 µL of a 5 pg/µL sample) as long as the total mass per assay tube is not less than the lower specification equivalent.

Example Maximum Kit Concentration:

Using 10 µL of the highest initial sample with a stated upper limit of 25 ng/µL results in a maximum absolute dsDNA mass of 250 ng in 200 µL. This can also be stated as 1.25 ng/µL after dilution in the assay tube.

Note: Higher initial sample concentrations may be measured by decreasing the volume (e.g., 1 µL of a 250 ng/µL sample) as long as the total mass per assay tube is not more than the upper specification equivalent.

Summary

The DeNovix suite of dsDNA Fluorescence Assays include three highly-specific, highly-sensitive kits for dsDNA quantification. Assay protocols are optimized for easy 2 Point Standard Curves, but they also offer the flexibility of using multi-point standard options. Assays are available in evaluation and larger sizes.

DeNovix RNA Assay Performance Data

The DeNovix RNA Assay enables highly specific fluorescence quantification of RNA over a broad range of concentrations using a single assay. The assay measures sample concentrations of 250 pg/µL – 1500 ng/µL RNA. This corresponds to a total mass of 0.5 ng – 1500 ng RNA.

The assay dye has excitation/emission maxima of 634/671 in the presence of RNA and is selective for RNA over dsDNA, ssDNA, and protein. The assay is compatible with fluorescence microplate readers and fluorometers with the appropriate excitation sources and emission detectors. This technical note presents typical performance data for the DeNovix RNA Assay measured using the fluorometer mode of the DS-11 Series instruments.

Basics of Absorbance Measurements

DeNovix RNA Assay:

A series of dilutions of *E. coli* total RNA was prepared in DEPC water. The assay working solution was prepared by mixing 4 mL of the assay buffer with 20 µL of the dye. For each sample, 190 µL of the working solution was added to a thin-walled, clear UV-transparent 0.5 mL PCR tube (DeNovix cat #TUBE-PCR-0.5-500). 10 µL of RNA was added to each tube for samples with concentrations between 0.5 ng/µL and 100 ng/µL. For the samples with a concentration less than 0.5 ng/µL, volumes were adjusted to use 180 µL working solution with 20 µL of RNA. Measurements of concentrations above 100 ng/µL were made using volume adjustments so that the total mass did not exceed 1500 ng (e.g. 3 µL of 500 ng/µL in 197 µL of working solution). Reaction solutions were mixed and incubated at room temp for 10 minutes while protected from ambient light. Five replicate measurements of each sample were taken to assess linearity.

The linear response of measured RNA as a function of expected concentration in the full linear range is presented in Figure 1. The range of the assay can successfully be extended past both the upper and lower limits, and extension has impact on linearity.

Qubit™ Broad Range (BR) and High Sensitivity (HS) RNA Assays

A Qubit™ 4 Fluorometer was used to test the same samples as the DeNovix RNA Assay with the Qubit™ BR and HS kits to minimize dilution differences. The Qubit™ BR kit (Q10210) (Lot #2086147) was tested across the published range of 20 ng – 1000 ng. Likewise, the Qubit™ HS kit (Q32852) (Lot #2113240) was tested over the published range of 5 ng – 100 ng. All manufacturers instructions for sample handling were followed for each kit.

Comparison with Qubit™ RNA Assays

The DeNovix RNA Assay covers a greater dynamic range than the combined range of the Qubit™ BR RNA and Qubit™ HS kits. Figure 1 compares the DeNovix RNA Assay dynamic range to the Qubit™ BR and Qubit™ HS RNA assays.

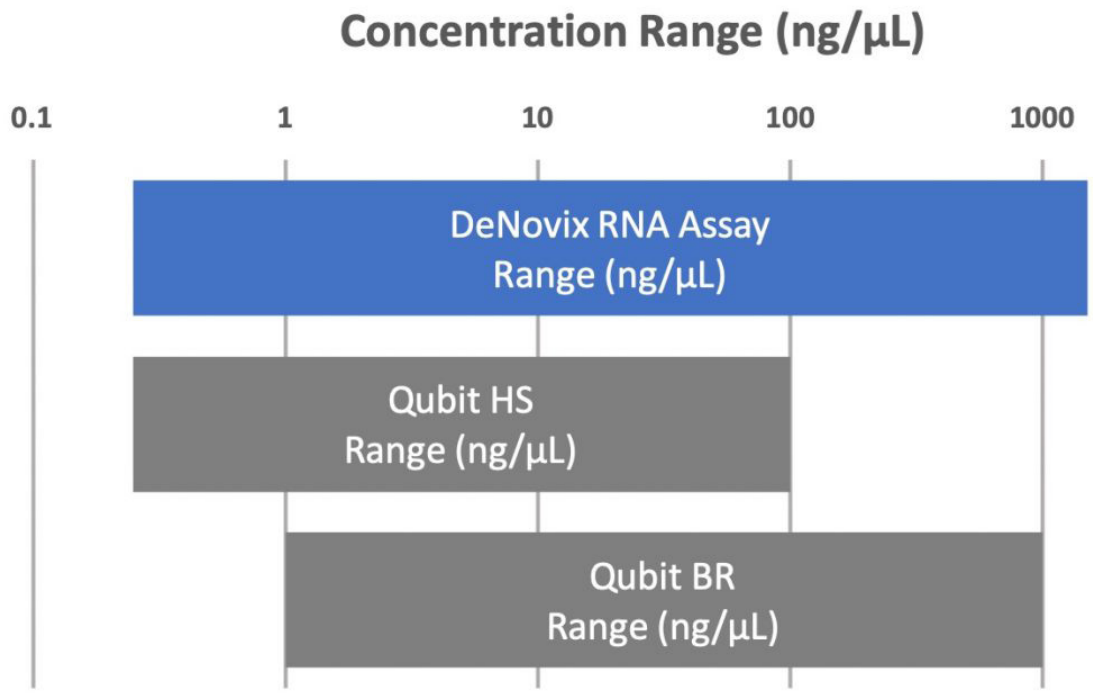


Figure 1. Dynamic Range: A comparison of Qubit™ RNA and DeNovix RNA Assays.

Performance Results

Figures 2 and 3 below demonstrate that the DeNovix RNA Assay enables measurement of RNA through a range of 0.5 ng (20 µL of 0.25 ng/µL) – 1500 ng (1 µL of 1500 ng/µL) by varying mass and volume of samples. The assay performance is linear over the range analyzed with an exceptional R2 value.

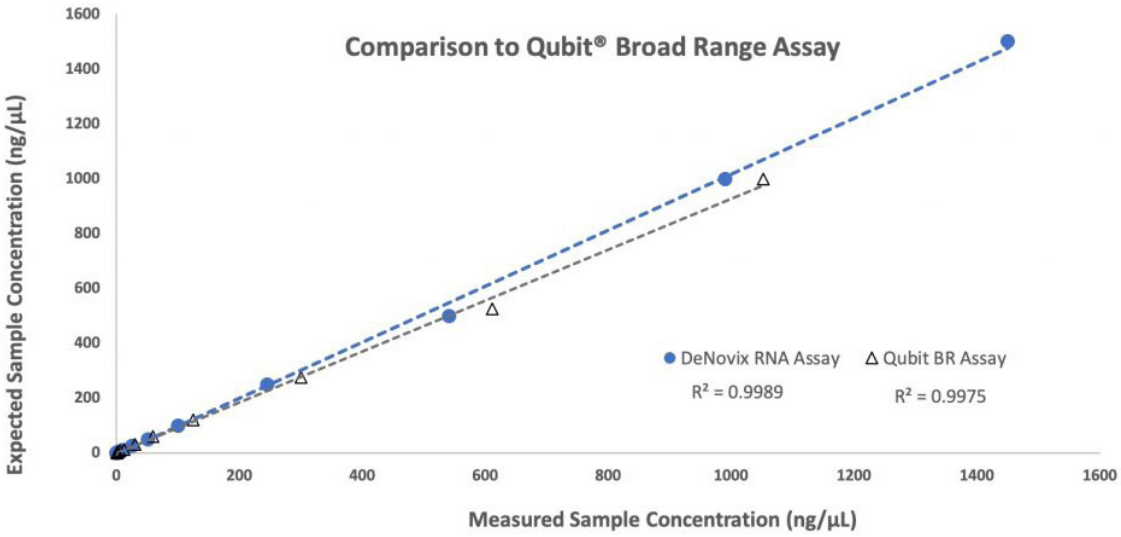


Figure 2. Comparison of Qubit™ BR RNA and DeNovix RNA Assay: A comparison of the Qubit™ BR RNA and DeNovix RNA Assay including linearity.

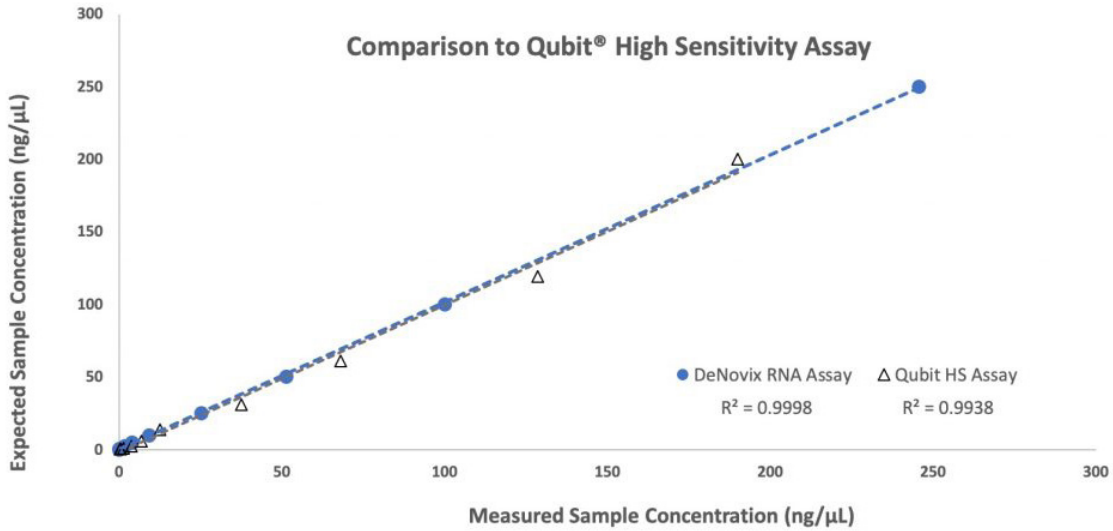


Figure 3. Comparison of Qubit™ HS RNA and DeNovix RNA Assay: A comparison of the Qubit™ HS RNA and DeNovix RNA Assay including linearity.

Table 1 lists the performance data that has been graphed in Figures 2 and 3. Five replicates were taken for each sample. The values in the expected category are based upon spectrophotometric analysis of each dilution to minimize the influence of pipetting errors.

Table 1. DeNovix RNA Assay performance data vs. Qubit™ BR and HS Quantification Assays.

RNA Assay Performance Data						
Expected	DeNovix Assay measured on DS-11 FX+		Thermo Fisher Qubit™ BR Assay measured on Qubit™ Fluorometer		Thermo Fisher Qubit™ HS Assay measured on Qubit™ Fluorometer	
ng/µL	ng/µL	%CV	ng/µL	%CV	ng/µL	%CV
1500	1449.879	0.772	Out of Range	Out of Range	Out of Range	Out of Range
1000	990.358	0.426	1052	3.454	Out of Range	Out of Range
500	520.106	0.272	610.8	2.888	Out of Range	Out of Range
250	245.554	2.556	299.6	1.284	Out of Range	Out of Range
100	100.001	0.319	124.52	0.070	128.48	5.345
50	51.460	1.084	59.44	1.075	68.12	3.496
25	25.361	1.454	29.68	1.022	37.52	1.752
10	9.309	2.304	12.572	0.965	12.48	1.827
5	4.051	1.281	5.964	1.286	6.784	2.914
2.5	1.770	0.617	1.496	1.386	3.716	1.841
1	0.683	1.072	1.208	2.061	1.382	1.939
0.5	0.362	2.625	0.698	3.844	0.474	38.771
0.25	0.160	3.483	Out of Range	Out of Range	0.334	2.445
0	0	0	Out of Range	Out of Range	Out of Range	Out of Range

Summary

The DeNovix RNA Assay enables specific, highly sensitive RNA quantification across a wide dynamic range. Compared to Qubit™, the DeNovix RNA assay requires only a single kit to cover a wider concentration range with highly linear, reproducible data. Researchers benefit from a greater proportion of samples fitting within the assay limits, decreasing the requirement to dilute or concentrate samples and eliminating the time and expense of repeating assays.

DeNovix Fluorescence Assays

DeNovix offers a range of fluorescence quantification assays for DS-11 Series Fluorometers and other compatible instruments. All assays offer sensitive and specific quantification for either dsDNA or RNA samples in the presence of contaminants.

DeNovix Fluorescence Assay	Range
dsDNA Broad Range	0.1 – 2000 ng/µL
dsDNA High Sensitvity	5 pg/µL – 250 ng/µL
dsDNA Ultra High Sensitivity	0.5 – 300 pg/µL
RNA Assay	0.25 - 1500 ng/µL

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