
**Technical Note 175**

# Microvolume Reproducibility Tips

## Introduction

DeNovix DS-11 Series Spectrophotometer / Fluorometer instruments enable the accurate and precise microvolume quantification of biomolecules. As with all microvolume techniques, it is important to apply best practices to ensure reliable results and reduce user error. Reproducibility of results is an important metric and is a good indication of data quality.

Issues with reproducibility are most often caused by either insufficient sample homogeneity or sample concentrations that are near the lower detection limit. This technical note is presented as a reference for solving issues with reproducibility of microvolume measurements.

## Sample Homogeneity

Well-mixed and homogeneous samples provide the most reproducible results. Sample homogeneity is especially important when using a microvolume method. Samples can start to settle even after a few minutes. This settling can result in a gradient of concentration within a sample tube where the concentration of an aliquot taken from the top of the sample is different from the concentration of an aliquot taken from the bottom of the sample. This can be more apparent in samples with larger molecules, such as genomic DNA.

Ensure that samples are vortexed or well-mixed immediately prior to each measurement. Some samples may need to be warmed to ambient temperature or heated to go completely into solution. Refer to Technical Note 106 for additional best practices recommendations.

## Concentrations Near the Spectrophotometer Limit of Detection

All spectrophotometers have a detectable level of background noise contributed by the environment and electronic components. The Lower Detection Limit of an instrument can be defined as the lowest quantity of analyte that can be distinguished from the background noise. Closer to this lower detection limit, the tolerance specification allows a greater percent CV than at higher concentrations.

## Tolerance Specifications

A tolerance specification may be given as either a percentage value or as a concentration for a specific analyte. For example, when the tolerance specification is 0.75 ng/µL for dsDNA, a sample with a concentration of 4 ng/µL could range from 3.25 to 4.75 ng/µL due to the contribution from electrical noise, as shown in Table 1. Note that the DS-11 Series has an industry-leading lower detection limit for dsDNA of 0.75 ng/µL.

Table 1: Tolerance Specifications for Microvolume Absorbance Measurements on DS-11 Series Spectrophotometer / Fluorometer

| Target Concentration (ng/µL dsDNA) | Tolerance Specification (ng/µL dsDNA) | Maximum % Error |
|---|---|---|
| 1000 | +/- 10 | 1% |
| 200 | +/- 2 | 1% |
| 100 | +/- 1 | 1% |
| 10 | +/- 0.75 | 7.5% |
| 4 | +/- 0.75 | 18.75% |
| 2 | +/- 0.75 | 37.5% |
| 0.75 | +/- 0.75 | 100% |
| 0.0 | +/- 0.75 | N/A |

## Summary

The most common causes of reproducibility issues are related to either non-homogeneous samples or to the concentration of samples being near the spectrophotometer lower detection limit. If the sample concentration is too low, the cuvette mode of DS-11 Series Spectrophotometer /

Fluorometer instruments extends the measurable concentration range to as low as 0.04 ng/µL. Fluorescence mode further enables quantitation of samples as low as 0.5 pg/µL dsDNA when using the DeNovix Ultra High Sensitivity Assay.

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