# Sample Reproducibility

Sampling from non-homogeneous solutions, particularly when using small volumes, may cause significant measurement deviation in the data generated using all measurement technologies including spectrophotometry . Genomic DNA, lambda DNA and viscous solutions of highly concentrated nucleic acids are common examples of sample types that require careful attention to ensure homogeneity before sampling. Proteins are subject to denaturation, precipitation, and aggregation and therefore also require special handling to ensure homogeneity before sampling. If results obtained using one of the Thermo Scientific NanoDrop spectrophotometers seem inaccurate or not reproducible, it is most likely the result of sample non-homogeneity or liquid column breakage (pedestal measurements).

## Suggestions

- **Ensure sample solution is homogeneous**
  Due to the small volumes required for pedestal measurements using a NanoDrop™ spectrophotometer, it is extremely important to ensure that the sample being measured is homogeneous. Field experience has shown that samples containing large molecules such as genomic or lambda DNA are particularly susceptible to this phenomenon. If compatible with the protocol being used, heating the DNA samples to 63°C and lightly vortexing before measurement may ensure the nucleic acid is truly in solution. Always refer to the specific protocol for suggestions to ensure all analyte is in solution.

- **Confirm sample is not too dilute**
  In general, measuring samples at or near the detection limit may result in higher CV values between replicates. Refer to the section on measurement concentration ranges in the specific NanoDrop spectrophotometer user manual for more information on the dynamic range for the application of interest.

- **Confirm that the reference (blank) solution and sample solvent are the same material**
  To minimize absorbance contribution of the blanking solution, it is recommended that the solution used to make a blank measurement be of the same pH and ionic strength as that used to re-suspend the unknown samples.

- **Use a 1.5-2 ul sample size for pedestal measurements**
  Erroneous results may occur when the liquid sample column is not completely formed during a measurement. While making a measurement, visually confirm that the liquid column is formed. If necessary, try a 1.5-2 ul sample size to ensure the column is formed. Concentration calculations are volume independent. It is recommended that spectrophotometric measurements be made immediately after pipetting samples onto the pedestal as delays can compromise accuracy.

- **Use fresh aliquots for each pedestal measurement**
  Multiple measurements of the same aliquot will result in evaporation and increased sample concentration values. This could lead to measurement reproducibility being out of specifications.

- **Make sure that the measurement sample surfaces are clean before starting the software modules**
  Dirty sample pedestals may cause erroneous absorbance readings (even negative values) and signal saturation. It is always good practice to first clean the sample surfaces with de-ionized water to remove any dried sample that might be present.

- **Re-condition the pedestals**
  Proteins and solutions containing surfactants are known to “un-condition” the measurement pedestal surfaces which may inhibit proper liquid column formation of subsequent samples. Use the instrument reconditioning kit, PR-1, as a rapid means of reconditioning the pedestals when the surface properties have been compromised and the liquid column breaks.

- **Confirm instrument accuracy with CF-1**
  CF-1 is a concentrated potassium dichromate calibration standard available from Thermo Fisher Scientific and its distributors. It is recommended that the calibration check procedure be performed every six months with a fresh vial of CF-1 to confirm that the instrument is performing within specifications.



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