

## Reproducibility Studies

### Introduction

The precision or reproducibility of the Thermo Scientific NanoDrop™ 1000 Spectrophotometer was assessed in three separate studies. The RNA and DNA studies utilized the absorbance at 260nm to automatically calculate the respective nucleic acid concentrations. The protein study utilized the absorbance at 280 nm.

### Method-RNA

tRNA was serially-diluted with distilled water to generate solutions that spanned the dynamic detection range of the instrument. Following blanking with water, one microliter (1ul) aliquots of the tRNA solutions were dispensed on to the spectrophotometer platform and measured in the Nucleic Acid Sample window using the RNA-40 setting. Spent sample was wiped from the pedestal with a Kimwipe. Ten individually-dispensed replicates were measured for each of the eight tRNA solutions as shown in Table 1.

| Sample      | ng/ul       | Sample | ng/ul       | Sample | ng/ul        | Sample | ng/ul       | Sample | ng/ul      | Sample | ng/ul        | Sample | ng/ul         | Sample | ng/ul         |
|-------------|-------------|--------|-------------|--------|--------------|--------|-------------|--------|------------|--------|--------------|--------|---------------|--------|---------------|
| 3200x       | 26.1        | 1600x  | 53.8        | 800x   | 110.1        | 400x   | 199.2       | 200x   | 441.6      | 100x   | 840.4        | 50*x   | 1663.2        | 25x    | 3161.4        |
| 3200x       | 25          | 1600x  | 54.5        | 800x   | 111.1        | 400x   | 201.2       | 200x   | 446.4      | 100x   | 836.8        | 50*x   | 1669.2        | 25x    | 3173.3        |
| 3200x       | 25.2        | 1600x  | 55.1        | 800x   | 113.7        | 400x   | 199.2       | 200x   | 456.8      | 100x   | 841.2        | 50*x   | 1689.2        | 25x    | 3156.2        |
| 3200x       | 24.6        | 1600x  | 54.9        | 800x   | 111.4        | 400x   | 226.4       | 200x   | 447.6      | 100x   | 839.6        | 50*x   | 1676.4        | 25x    | 3179.1        |
| 3200x       | 24.2        | 1600x  | 55          | 800x   | 110.9        | 400x   | 222.8       | 200x   | 447.6      | 100x   | 835.6        | 50*x   | 1722.4        | 25x    | 3158          |
| 3200x       | 24.7        | 1600x  | 55.6        | 800x   | 111.2        | 400x   | 230.7       | 200x   | 444.4      | 100x   | 839.2        | 50*x   | 1684.4        | 25x    | 3155.1        |
| 3200x       | 25.5        | 1600x  | 54.7        | 800x   | 111.3        | 400x   | 233.4       | 200x   | 448        | 100x   | 834          | 50*x   | 1673.6        | 25x    | 3148.7        |
| 3200x       | 25.7        | 1600x  | 54.3        | 800x   | 111.8        | 400x   | 229.3       | 200x   | 446        | 100x   | 851.2        | 50*x   | 1685.6        | 25x    | 3156.7        |
| 3200x       | 25.8        | 1600x  | 54.9        | 800x   | 111          | 400x   | 228.9       | 200x   | 448.8      | 100x   | 838.8        | 50*x   | 1686          | 25x    | 3157.9        |
| 3200x       | 26.2        |        |             | 800x   | 111.5        | 400x   | 229         | 200x   | 452.4      | 100x   | 835.2        | 50*x   | 1692          | 25x    | 3167.3        |
| <b>MEAN</b> | <b>25.3</b> |        | <b>54.7</b> |        | <b>111.4</b> |        | <b>220</b>  |        | <b>448</b> |        | <b>839.2</b> |        | <b>1684.2</b> |        | <b>3161.4</b> |
| <b>SD</b>   | <b>0.7</b>  |        | <b>0.5</b>  |        | <b>0.9</b>   |        | <b>14.2</b> |        | <b>4.2</b> |        | <b>4.8</b>   |        | <b>16.3</b>   |        | <b>9.2</b>    |

### Method-DNA

A second reproducibility study was conducted using three different concentrations of Herring sperm DNA diluted in TE buffer. All of the DNA solutions were measured using the 1.0mm pathlength since they were < 500ng/ul. Following Blanking with TE buffer, 1ul aliquots of the DNA sample solutions were measured in the Nucleic Acid Sample window using the DNA-50 setting. Spent sample was wiped from the pedestal with a Kimwipe. Ten replicates at the three DNA concentrations, plus water, were individually measured by dispensing with a multi-dispense pipettor. Data shown in Table 2 on the next page.

Table 2.

| Sample      | ng/ul        | Sample  | ng/ul       | Sample   | ng/ul        | Sample    | ng/ul         |
|-------------|--------------|---------|-------------|----------|--------------|-----------|---------------|
| water       | 0.27         | 3 ng/ul | 4.32        | 33 ng/ul | 36.2         | 333 ng/ul | 305.18        |
| water       | 0.2          | 3 ng/ul | 3.88        | 33 ng/ul | 36.36        | 333 ng/ul | 306.36        |
| water       | -0.22        | 3 ng/ul | 4.66        | 33 ng/ul | 35.18        | 333 ng/ul | 305.76        |
| water       | -0.79        | 3 ng/ul | 4.46        | 33 ng/ul | 35.89        | 333 ng/ul | 305.82        |
| water       | -0.76        | 3 ng/ul | 4.52        | 33 ng/ul | 35.49        | 333 ng/ul | 304.83        |
| water       | -0.34        | 3 ng/ul | 3.28        | 33 ng/ul | 35.94        | 333 ng/ul | 304.64        |
| water       | -0.78        | 3 ng/ul | 2.98        | 33 ng/ul | 35.58        | 333 ng/ul | 307.05        |
| water       | -0.11        | 3 ng/ul | 3.63        | 33 ng/ul | 35.13        | 333 ng/ul | 307.83        |
| water       | -0.22        | 3 ng/ul | 2.8         | 33 ng/ul | 36.8         | 333 ng/ul | 309.97        |
| water       | 0.34         | 3 ng/ul | 3.28        | 33 ng/ul | 35.8         | 333 ng/ul | 309.56        |
| <b>MEAN</b> | <b>-0.24</b> |         | <b>3.78</b> |          | <b>35.84</b> |           | <b>306.70</b> |
| <b>SD</b>   | <b>0.43</b>  |         | <b>0.68</b> |          | <b>0.52</b>  |           | <b>1.89</b>   |

## Method-Protein

The Absorbance of a protein solution (A-1), measured at 280nm on two NanoDrop 1000s and normalized at 340nm in the UV-Visible, is shown in the table 3 below. The Absorbance of a second protein solution (B-1), was also measured at 280nm, normalized at 340nm in the UV-Visible window, and is shown in table 4.

Table 3.

| Sample ID   | A280nm       | A280nm       |
|-------------|--------------|--------------|
| Protein A-1 | 0.094        |              |
| Protein A-1 | 0.08         |              |
| Protein A-1 | 0.085        | 0.079        |
| Protein A-1 | 0.082        | 0.077        |
| Protein A-1 | 0.071        | 0.078        |
| Protein A-1 | 0.083        | 0.073        |
| Protein A-1 | 0.071        | 0.073        |
| Protein A-1 | 0.072        | 0.076        |
| Protein A-1 | 0.072        | 0.071        |
| Protein A-1 | 0.071        | 0.07         |
| <b>MEAN</b> | <b>0.078</b> | <b>0.074</b> |
| <b>SD</b>   | <b>0.008</b> | <b>0.003</b> |

Table 4.

| Sample ID   | A280nm       |
|-------------|--------------|
| Protein B-1 | 0.997        |
| Protein B-1 | 0.985        |
| Protein B-1 | 0.986        |
| Protein B-1 | 0.982        |
| <b>MEAN</b> | <b>0.988</b> |
| <b>SD</b>   | <b>0.007</b> |