

ND-1000 Linearity Studies

Introduction

The linear response of absorbance with concentration was evaluated in four separate studies. The NanoDrop® ND1000 was compared with two commercial UV-Visible spectrophotometers fitted with a 2mm pathlength, quartz micro-cuvette.

A comparison of spectrophotometers, restricted to the linear absorbance range of the HP8452A using a 2 mm micro-cuvette is shown in figure 2.

Method– Herring Sperm DNA

Dilutions of commercial herring sperm DNA (~10 mg/ml) were prepared using Tris-EDTA (TE) buffer, pH 8.0. The respective DNA absorbances were measured at 260nm using an HP8452A UV-Visible Spectrophotometer and a NanoDrop® ND1000 Spectrophotometer as described (manual). DNA concentrations (ng/ul) for the HP were calculated by multiplying the respective A260nm values by 250 (2mm pathlength cuvette). Direct DNA concentration readouts from the NanoDrop® ND1000, for the respective dilutions, were taken from the instrument’s Nucleic Acid Sample screen or the printout.

Figure 2.

DNA Dilution: ND-1000 vs. HP8452A

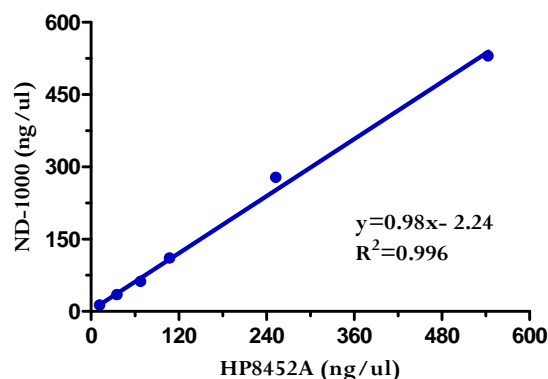
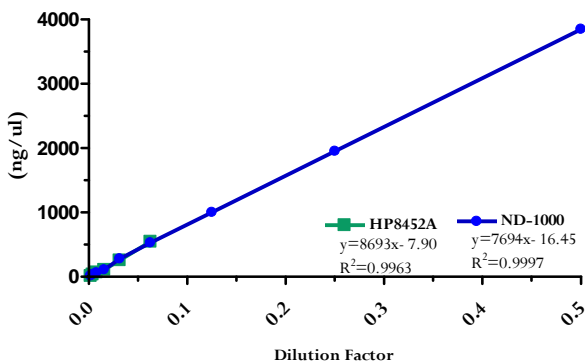


Figure 1.

DNA Linearity: Comparison of Two Fold Dilutions read on HP8452A and ND-1000

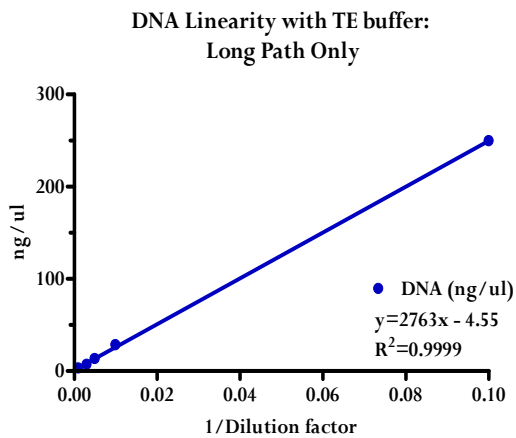
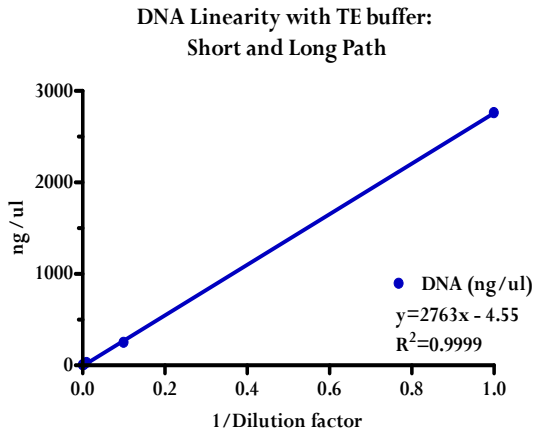


A second linearity study on the NanoDrop® ND1000 Spectrophotometer , again using herring sperm DNA in TE buffer, yielded the following results:

Dilution	1/dilution factor	DNA ng/ul
Initial concentration	1.000	2759.9
10 fold	0.100	249.74
100 fold	0.010	28.39
200 fold	0.005	13.49
400 fold	0.003	7.1
1000 fold	0.001	3.23

The linearity response of the HP8452A Reference Spectrophotometer using the 2 mm quartz microcuvette and the NanoDrop® ND1000 Spectrophotometer’s Nucleic Acid Sample window is shown in figure 1. The ND-1000 automatically calculates data obtained with both the 1.0 mm and 0.2 mm pathlengths. The same set of diluted DNA samples was used for both instruments.

The data from table 1 is graphed below:



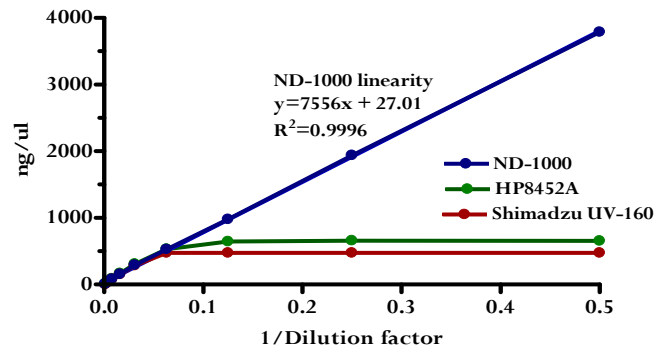
Method– Calf Thymus DNA

Calf thymus DNA (~10mg/ml) was serially diluted with water and the respective solutions were run on three UV-Visible Spectrophotometers: the NanoDrop® ND1000, an HP8452A, and a Shimadzu UV-160. In order to extend the dynamic linear range of the cuvette-requiring spectrophotometers, a 2 mm-pathlength, quartz cuvette requiring a minimum 10ul sample volume was used. The respective data is shown in table 2.

Table 2.

Dilution factor	HP8452A (ng/ul)	Shimadzu UV-160 (ng/ul)	NanoDrop ND1000 (ng/ul)
0.001	13.0	1.3	7.6
0.002	21.8	12.5	18.4
0.004	44.0	34.5	39.7
0.008	88.5	77.0	80.6
0.016	169.5	151.8	156.4
0.031	307.5	286.0	292.6
0.063	530.0	474.8	527.0
0.125	646.5	474.8	978.1
0.250	657.5	474.8	1938.1
0.500	655.0	474.8	3787.2

Spectrophotometer Comparison:
Calf Thymus DNA



The data for all three spectrophotometers across the entire DNA concentration range is graphed above.

Method– Protein

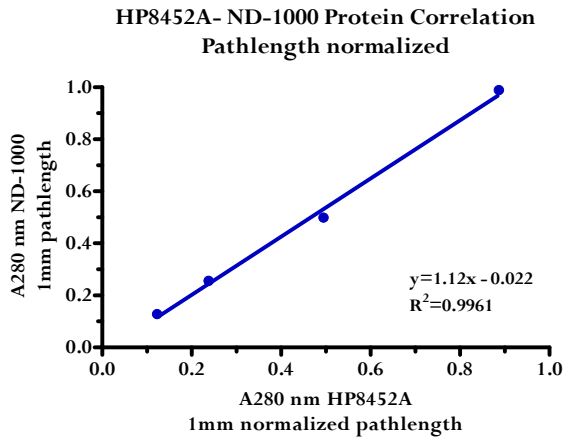
Serial dilutions of commercial protein (B-1, ~10 mg/ml) were prepared using water as diluent. The respective protein dilutions were measured at 280nm using an HP8452 Spectrophotometer with a 2 mm pathlength quartz microcuvette and a NanoDrop® ND1000 Spectrophotometer as described (UV-Visible window in manual). The respective values are shown in the table 3.

Protein concentrations for the_HP were calculated by multiplying the respective A280nm values (normalized at 340nm) by 5. Protein concentrations of the protein dilutions run on the NanoDrop® ND1000 were calculated by multiplying the respective A280nm (normalized at 340nm) values by 10. The average values are presented in the table.

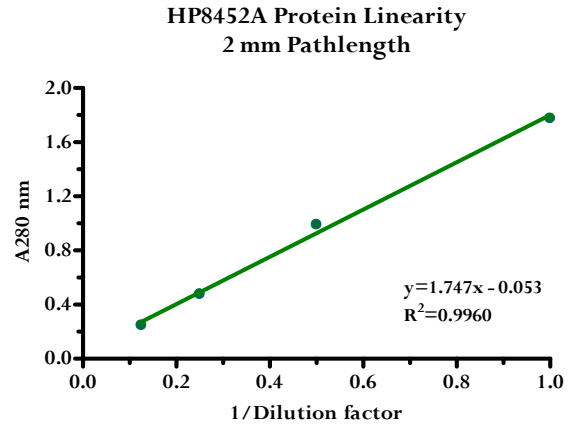
Table 3.

HP8452 (2mm)	HP8452 (1mm-norm)	ND-1000 (1mm)
0.247	0.124	0.126
0.478	0.239	0.254
0.991	0.496	0.497
1.776	0.888	0.987

A comparison of the spectrophotometers, normalized to a 1 mm pathlength setting, is shown in the graph below:



The response linearity of the HP8452 Spectrophotometer using the 2 mm quartz microcuvette and the serially-diluted protein samples is shown in the following graph:



The response linearity of the NanoDrop® ND1000 Spectrophotometer's UV-Visible window for the corresponding protein solutions is shown below:

