

# Determination of Plasmid DNA Purity Ratio in Human Gene Therapy Products Using the Slope Spectroscopy® Method

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## Abstract

Estimation of DNA purity in the presence of protein for gene therapy plasmid material is possible by exploiting the 260/280 UV absorbance ratio. This technique, however, has significant challenges when accurate quantification of DNA purity is required. The CTech™ SoloVPE® and the Slope Spectroscopy® method offer a new method to overcome these challenges, and to accurately measure DNA purity ratios in accordance with the Beer-Lambert law.

## Summary

Gene therapy is an exciting opportunity to change the way genetic diseases are treated. It has the potential to provide a lifelong solution to unmet medical needs. For these purposes, DNA purity is examined by testing the ratio of absorbance at 260 nm and 280 nm. This ratio is also known as the *R* value.

Nearly all traditional spectrophotometers rely on fixed-pathlength UV-Vis absorbance readings. Several issues can arise from using this method, especially when dealing with the impurities of plasmids. Common impurities in plasmid DNA (pDNA) include free nucleotides, single-stranded nucleic acids, and proteins. Proteins are composed of 20 amino acids; only four (tryptophan, tyrosine, cysteine, and phenylalanine) absorb light at 260 nm, and only three (tryptophan, tyrosine, cysteine) absorb light at 280 nm. The *R* value varies greatly from the abundance of the four amino acids that absorb light at 260 nm.

Also, nucleic acid absorbance is sequence dependent. Although the nucleic acids absorb at 260 nm and 280 nm, the amount of light they absorb at each wavelength is not the same. This

results in different extinction coefficients and *R* values by sequence.

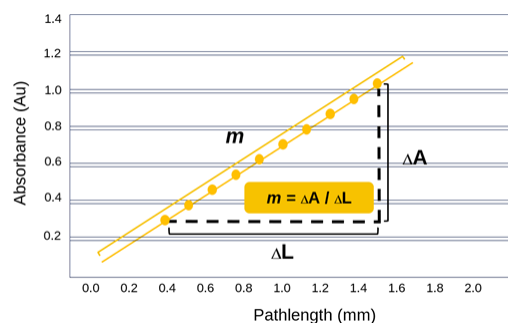
This application note demonstrates how the SoloVPE System can accurately measure the *R* value within plasmids. The Slope Spectroscopy method addresses users' challenges with sample volume and dilution and inconsistent wavelength readings, enabling the instrument to provide reliable data based on specified parameters within the software. Despite all the challenges, the SoloVPE System enables users to quickly and precisely measure absorbance readings at variable pathlengths.

## Apparatus/Equipment

Figure 1. SoloVPE System



Figure 2. Variable-pathlength UV-Vis absorbance readings



- SoloVPE instrument (Part No. SYS-VPE-SOLO5)
- Cary 60 UV-Vis spectrophotometer (Part No. IN-CARY60 or Agilent Part No. G6860A)
- CTech™ ConfiRM™ Slope Reference Materials, 1.999 Abs/mm (Part No. MRM-PFI08-01-P10)
- ConfiRM Standard, 4.975 Abs/mm (Part No. MRM-PFI08-02-P10)
- ConfiRM Standard high, 23 Abs/mm (Part No. MRM-01-P1)
- Insulin solution (Sigma-Aldrich SKU I0516-5ML)
- Plasmid pCl-neo vector (Lake Pharma - Project code 2019-1888, lot # MB2017459)
- CTech™ Fibrette® Optical Component (Part No. OF0002-P50)
- Disposable UV plastic vessels (Part No. OC0009-1-P50)

### Method and Results

The *R* value is generally accepted as “pure” if the ratio is between 1.8 and 2.0. The ConfiRM standard was used to effectively demonstrate this acceptance criteria. At the start of analysis, each standard was measured in triplicate at 272 nm.

$$R = \frac{\text{Slope}_{260\text{ nm}} \left( \frac{\text{AU}}{\text{mm}} \right)}{\text{Slope}_{280\text{ nm}} \left( \frac{\text{AU}}{\text{mm}} \right)}$$

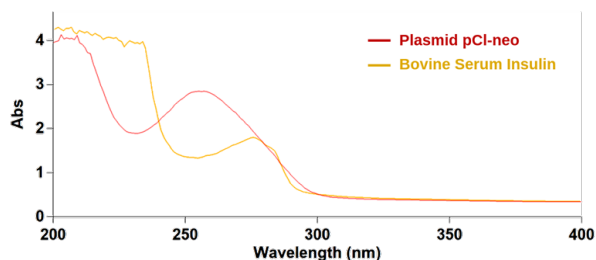
Stock solutions of 1.0 mg/ml pCl-neo DNA plasmid and 1.0 mg/ml bovine serum insulin protein were prepared by diluting with water. A spectral scan of the stock solutions were taken from 200 nm to 360 nm. The absorbance profiles are demonstrated in Figure 3.

System suitability testing was done with the SoloVPE System to verify stock concentration at 1.0 mg/ml. Extinction coefficients used were as follows:

$$\text{Plasmid DNA } \epsilon_{260\text{ nm}} = 20 \left( \frac{\text{mg}}{\text{ml}} \right)^{-1} \text{ cm}^{-1}$$

$$\text{Bovine serum insulin } \epsilon_{280\text{ nm}} = 0.965 \left( \frac{\text{mg}}{\text{ml}} \right)^{-1} \text{ cm}^{-1}$$

Figure 3. UV Profile of pCLS24044 plasmid and bovine insulin protein



After confirming that the stock solutions were at 1.0 mg/ml, 25 purity levels from 100% plasmid to 100% protein were determined. They can be observed in Table 1.

Table 1. Purity ratio levels

Level	% Protein	% DNA
1	100.0%	0.0%
2	97.5%	2.5%
3	95.0%	5.0%
4	92.5%	7.5%
5	90.0%	10.0%
6	85.0%	15.0%
7	80.0%	20.0%
8	75.0%	25.0%
9	70.0%	30.0%
10	65.0%	35.0%
11	60.0%	40.0%
12	55.0%	45.0%
13	50.0%	50.0%
14	45.0%	55.0%
15	40.0%	60.0%
16	35.0%	65.0%
17	30.0%	70.0%
18	25.0%	75.0%
19	20.0%	80.0%
20	15.0%	85.0%
21	10.0%	90.0%
22	7.5%	92.5%
23	5.0%	95.0%
24	2.5%	97.5%
25	0.0%	100.0%

Each purity level was analyzed by the SoloVPE System. Triplicate slope measurements were obtained at 260 nm and 280 nm and averaged for a single reportable slope value. The *R* value was calculated for each level by slope ratio.

### Analysis

The purity ratios obtained are plotted against their percent purity. The purity ratios obtained at each level correlate well with the theoretical ratio curve. The SoloVPE software provided the slope values at 260 nm and 280 nm simultaneously and then automatically calculated the ratio with the User Result feature.

**Table 2. Purity ratio accuracy**

Level	Theoretical Purity Ratio	Observed Purity Ratio	% Difference
1	0.6259	0.62723	0.21%
2	0.87087	0.90315	-0.22%
3	1.05311	1.06122	0.74%
4	1.18483	1.17076	-1.14%
5	1.28451	1.28847	0.30%
6	1.42528	1.42358	-0.11%
7	1.51996	1.52481	0.31%
8	1.58798	1.58959	0.10%
9	1.63927	1.65568	0.96%
10	1.67930	1.67489	-0.25%
11	1.71134	1.69203	-1.09%
12	1.73770	1.71515	-1.25%
13	1.75964	1.73533	-1.33%
14	1.77821	1.77064	-0.41%
15	1.79418	1.81956	1.36%
16	1.80804	1.77874	-1.56%
17	1.82026	1.80414	-0.85%
18	1.83094	1.81184	-1.00%
19	1.84046	1.82975	-0.56%
20	1.84902	1.85457	0.29%
21	1.85672	1.84667	-0.52%
22	1.86028	1.88378	1.22%
23	1.86365	1.85282	-0.56%
24	1.86692	1.84941	-0.90%
25	1.87	1.87147	0.08%

Calculating the purity ratio, a theoretical ratio was determined for each level. As the protein and DNA absorbance is non-interfering, the absorption of light by the combination of both chromophores is additive and may be calculated by using the following equation:

$$\text{Slope Ratio}_i = \sum \text{Slope Ratio}_{ij\phi_j}$$

- $\phi$  represents the volume fraction,
- $j$  represents the components (i.e., DNA or protein),
- $i$  represents the wavelengths (i.e., 260 nm or 280 nm).

The theoretical and observed *R* values and percent error are compiled into Table 2. From the 25 purity ratios obtained, the maximum observed error from theoretical ratio was 1.56%. The average observed error was 0.25%. These results demonstrate a great accuracy and reproducibility at variety of purity ratios with a less than 2% deviation for all 25.

### Conclusion

The current perception is that purity by UV 260/280 ratio is not useful, due to the availability of orthogonal assays with greater sensitivity. If values that exceed the typical variation of 1% are consistently observed, it could indicate impurity levels of ~15%. For a 1.0 mg/ml plasmid, this would indicate a protein contamination of 150 µg/ml, which is only slightly higher than the sensitivity of colorimetric techniques. The sensitivity is significantly improved with SoloVPE System over traditional spectrophotometers.

The SoloVPE System and its dedicated software allowed measurements to be taken simultaneously at 260 nm and 280 nm, with results automatically reported. SoloVPE UV spectroscopy for determination of plasmid DNA purity ratio has proven to be a critical analytical tool, expediting the testing of challenging samples, providing immediate feedback, and opening up multiple possibilities for meeting medical needs.

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