

## *Nucleic Acid $A_{260}/A_{280}$ Purity Test*

As a contract manufacturing organization, VGXI deals with many different types of customers. Regardless of what type of plasmid they are interested in having us manufacture, Quality Control (QC) testing is something that is on the minds of every single one of them. In particular, there is keen interest in the assessment of nucleic acid purity. One test for nucleic acid purity, known as the  $A_{260}/A_{280}$  test, is widely used for measuring the purity of both nucleic acids and proteins.

What is  $A_{260}/A_{280}$  and what does it mean? Well, nucleic acids and proteins have an absorbance maxima at 260nm and 280nm, respectively. By taking the ratio of the absorbance at these wavelengths, researchers are able to measure the nucleic acid purity of their product. A ratio of 1.8 to 2.0 is generally accepted as 'pure'. This absorption ratio is useful in assessing DNA contamination of protein solutions, since the aromatic amino acids of protein absorb light at 280 nm. However, using  $A_{260}/A_{280}$  to measure the purity of nucleic acids does have limitations, as it takes a relatively large amount of protein contamination to significantly affect the  $A_{260}/A_{280}$  in a nucleic acid solution.

Why use the  $A_{260}/A_{280}$  for nucleic acids as opposed to proteins? An  $A_{260}/A_{280}$  reading of a 100% nucleic acid solution is 2.00, whereas the same measurement of a 100% protein solution is 0.57. It should also be noted that the sensitivity of nucleic acid contamination is significant in protein solution. With 30% contamination of nucleic acid, the ratio increases to 1.73 from 0.57, whereas 30% contamination of protein in nucleic acid only reduces the ratio to 1.94 from 2.00. This difference is due to the higher extinction coefficient of nucleic acids at 260 nm and 280 nm, compared to that of proteins. There are several factors could affect the results of this test. Contaminants, such as residual phenol and other solutions associated with extraction, a low concentration (<10 ng/ $\mu$ l) of nucleic acid or a solution with an acidic pH could all contribute to a low ratio. Conversely, a high ratio is not necessarily indicative of an issue or impure product. Although purity ratios are important indicators of sample quality, the best indicator of DNA or RNA quality is functionality in the downstream application of interest. If the purity ratio is significantly higher than expected, it is best to review the spectral profile as a primary means of troubleshooting. While 260 nm measurements are made very near the peak of the absorbance spectrum for nucleic acids, the 280 nm measurement is located in the portion of the spectrum that has a very steep slope. As a result, very small differences in the wavelength in and around 280 nm will effect greater changes in the  $A_{260}/A_{280}$  ratio than small differences at 260 nm.

Different instruments could also result in slightly different  $A_{260}/A_{280}$  ratios on the same solution. This is mainly due to variability in wavelength accuracy between instruments. A  $\pm 1$  nm difference in wavelength accuracy could cause as much as a  $\pm 0.2$  change in  $A_{260}/A_{280}$ . With a claim of  $\pm 1$  nm accuracy, it is possible to have as much as  $\pm 0.4$  difference in  $A_{260}/A_{280}$  between two instruments.

While it is widely accepted as a standard QC test used in the plasmid community,  $A_{260}/A_{280}$  testing was never meant to offer researchers a precise answer regarding the purity of their product. We always recommend comprehensive QC testing to ensure that our customers can be assured that the products they receive from VGXI are of the highest quality.

## **About VGXI**

VGXI is a contract manufacturing organization that specializes in the production of DNA plasmids under GMP for human clinical trials. With over 13 years' experience, we have produced products for numerous clinical trials in the U.S., Europe and Asia. Our patented AIRMIX® manufacturing technology produces the highest quality plasmids in the industry with plasmid supercoil percentages up to 99% and final product concentrations as high as 15 mg/ml. Our highly experienced team can work with your project's unique requirements to create a manufacturing solution that ensures the success of your clinical program.



**2700 Research Forest Dr. Suite 180  
The Woodlands, TX 77381**