APPLICATION NOTE

No. T090

Using the NanoDrop 3300 Fluorospectrometer

For nanoparticle/quantum dot applications

Introduction

Nanoparticle research is a broad area of growing scientific interest because of its potential relevance to biomedical and other diverse fields. Potential applications in medicine have led to advances in coating the core's outer layers with polymers or bio-functional groups.[1, 2] Quantum dots (QD), visible-IR emitting nanoparticles, are used as fluorescent probes and have significant potential for molecular detection, bioimaging and diagnostics in biomedicine.[1, 3] Often, researchers working in nanoparticle studies need to make fluorescence measurements; many different methods are employed for this purpose. Here we discuss how a unique instrument, the Thermo Scientific™ NanoDrop™ 3300 Fluorospectrometer, is increasingly being used for a wide variety of such nanoparticle fluorescence applications, because of its simplicity, versatility and microvolume capability.

The NanoDrop 3300 Fluorospectrometer uses a patented "cuvette-less" sample retention system for fluorescent measurements using as little as one microliter sample volume. With UV, blue and white LED excitation, it covers a broad wavelength range, thereby facilitating the use of many diverse fluorophores; it is also able to analyze multiple emission profiles from a single sample. The following examples describe some of the uses of NanoDrop microvolume fluorescence technology as it pertains to nanoparticle research.

DNA methylation detection

DNA methylation is an important regulator of gene transcription, and the role of DNA methylation in carcinogenesis has been an increasing area of focus in the field of epigenetics over the last decade. Alterations in DNA methylation patterns are common in a variety of tumors and also play an essential role in cellular development. Methylation sensitive PCR (MSP) is a commonly used technique to assay for methylation detection. MSP reactions consist of several cycles of PCR and may require an additional round of amplification.

Recently, a new technique, methylation-specific quantum dot fluorescence resonance energy transfer (MS-qFRET), has been developed. MS-qFRET is a faster and more sensitive technique than MSP for detecting minute amounts of methylated DNA in the presence of a 10,000 fold excess of unmethylated alleles. In order to measure fluorescent probes, scientists often use high-end spectrofluorometers, which can be very expensive and complicated, or resort to relatively awkward and unreliable lab-built set-ups using microscopes and CCD cameras. In a progressive approach, Bailey *et al.* successfully used the NanoDrop 3300 Fluorospectrometer to measure QD 605-Cy5 FRET probes in proof of concept experiments, as well as in the method development and implementation of the new technique.[4, 5]

DNA-nanoparticle development

DNA hairpins bound to carbon nanotubes have been used to demonstrate the potential of nanostructuring without affecting the electronic properties of the nanotubes.[6] Probing of the nanotube-hairpin DNA structures was achieved using either complementary or non-complementary DNA sequences attached to FAM, followed by filtration. Binding of the FAM-labeled DNA probe to the nanotube-DNA hairpin structure resulted in the presence of fluorescence in the filtered nanotubes. It was also shown that QD bound to connector splints could be used to link to specific or non-specific oligonucleotides. Müller *et al.* incorporated the NanoDrop 3300 Fluorospectrometer into their workflows to detect FAM or QD fluorescence signals either free or bound to nanotubes.

Antigen detection

Developers of diagnostic tests strive to reduce assay cost, complexity and time without compromising accuracy. Conventional methodologies such as ELISA, gel electrophoresis, and protein microarrays are all limited by one or more of these factors. An alternative method of protein detection employs antibody-QD conjugates with flow cytometry to detect the binding of specific antigens.

Soman *et al*. used a streptavidin-biotin interaction to conjugate QD 525, QD 585 and QD 705 to different antibodies.[7] Conjugate construction was monitored using a NanoDrop 3300 Fluorospectrometer before antigen detection via flow cytometry. Selection of this cost-effective solution was based primarily on its micro-volume capability, which is important for downstream sample conservation.

In a similar study, Jayagopal *et al*. used QD 585, QD 655 and phycoerythrin as vascular imaging agents after PEG-based conjugation to antibodies.[8] The NanoDrop 3300 was used in this study in order to determine fluorescence intensity of different antibody–QD conjugates prior to flow cytometry and immunofluorescence microscopy analysis. Once again, the small volume capability makes the NanoDrop 3300 Fluorospectrometer ideal in this case because quantification of antibody-QD fluorescence must be accomplished with minimal loss of sample.

Cancer therapy and drug delivery

Successful targeting of tumor cells has the potential to raise the efficacy of cancer treatments by limiting or eliminating toxicity to normal tissues caused by traditional systemic chemotherapeutic or radiation treatment. The engineering of QDs with oncophillic properties that provide such targeted specificity is currently in progress.

In work by Sewell *et al*., QD 585 was conjugated to both folic acid and 5-FAM, with the 5-FAM link made using the MMP-7 cleavable peptide.[9] In the presence of tumor cells, MMP-7 proteases cleaved the MMP-7 peptide linking QD 585 and 5-FAM, resulting in loss of 5-FAM fluorescence signal. The change in signal was monitored using the NanoDrop 3300 Fluorospectrometer.

In addition to specificity, another key requirement of successful nanoparticle therapeutic delivery systems is the continued stability of the molecular components en route to and after cellular uptake. Maintenance of activity is important in understanding the effects of a cell modulator, vaccine, or poison being delivered. The therapeutic delivery study conducted by DeLong *et al*. investigated whether or not the presence of protamine aided in stabilizing DNA molecules bound to gold nanoparticles.[10] In this study, DNA stability was assayed using Hoechst dye and the NanoDrop 3300 Fluorospectrometer.

Summary

The NanoDrop 3300 Fluorospectrometer has played an integral role in relevant and diverse nanoparticle research applications. In these cases, the justifications for its use are equally diverse.

In some studies, sample conservation is the key driver. In others, sample availability is a non-issue, but having a cost-effective, simple, versatile fluorescence instrument is most important. This unique ultra-small footprint instrument measures micro-volume samples and offers convenience with pre-configured methods for common fluorophores; but is also flexible enough to be able to customize user-created methods and handle a wide range of fluorophores. It is allowing researchers to make their fluorescence measurements simply, quickly and cost-effectively, making it a valuable tool for nanoparticle research and biomedical applications.

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