



Bioprocessing Tutorial

Use of Microvolume Analysis in Processing

NanoDrop 8000 Was Designed to Reduce Complexity and Streamline Bioproduction

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Simple solutions are increasingly being integrated in the ever-changing bioprocessing environment. As methodologies improve throughout the protein production industry, tools must have the flexibility to adapt quickly to new workflow demands. With process analytical technology (PAT) continuing to bring significant improvements, innovative technologies that can evolve with industry methodologies are essential for bioprocess optimization. The choice of equipment and instrumentation has a great impact on the protein process workflow and on de-bottlenecking steps.

One example of integration of a simple solution is the use of microvolume instrumentation for quality-control testing in Diosynth Biotechnology's (www.diosynthbiotechnology.com) protein processing facility. As the demand to reduce the cost of biotech drugs increases, there is a growing

emphasis on how companies like Diosynth perform bioprocessing more effectively, including Quality by Design (QbD) and PAT initiatives.

Microvolume Quality Control

The implementation of microvolume UV testing at-line eliminates batch testing, greatly reducing processing time, and increasing efficiency. Microvolume UV analysis is performed using Thermo Scientific NanoDrop™ 8000 spectrophotometers (www.nanodrop.com). Each spectrophotometer measures multiple protein samples using a sample-retention system that requires small amounts (~2

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µL) of protein sample. The system uses the inherent physical properties, namely surface tension, to hold samples in place during measurement, eliminating the need for cuvettes, capillaries, or other containment devices.

Optical pedestals hold and measure up to eight microvolume protein samples at a time. The removal of classic containment devices allows the path length between the optical pedestals to



Figure 1. The sample-retention system of the NanoDrop 8000 uses surface tension to hold and measure up to eight 1 µL protein samples at a time.

change during the measurement cycle. The ability to optimize path length results in an extensive dynamic range of possible protein concentrations that can be measured (e.g., 0.1 mg/mL to 120 mg/mL for HSA), essentially eliminating the need to perform time-consuming dilutions and the errors associated with preparing such dilutions (*Figure 1*).

Protein Manufacturing

Protein concentration monitoring is important for determining the step yield, as well as the capacity and efficiency of a particular protein production run. Downstream processes such as protein purification and buffer exchange for final formulations require accurate protein quantitation for quality control. Previously, batch testing was performed using standard cuvette methodology. Multiple cuvette volumes of product needed to be pulled from the manufacturing line and sent to another area for QC analysis. Results were then sent back to the manufacturing floor to calculate the step yield, determine the amount of product needed for subsequent steps, and make any adjustments to ensure the system reaches a desired target amount.

The integration of a microvolume analysis system at Diosynth has provided increased confidence in the processing results and fidelity of the end product for several reasons. First, the molecular integrity of the sample remains intact during testing, which is highly preferred from a regulatory standpoint. Second, microsampling reduces waste and allows real-time adjustments to be made immediately at the processing line. Third, the multisample capability of the microvolume UV-Vis system allows for convenient replicate sampling, providing a broader data set for

quality assurance. Finally, having a common platform for sample testing facilitates training and integration of this technology throughout a facility.

Microvolume testing allows the iterative process between production and quality control to take place on the protein manufacturing floor in real time. The eight-pedestal system of the NanoDrop 8000, with a measurement time of ~15 seconds for eight samples, allows a large set of data to be generated, which, in turn, provides greater confidence in the condition of the product batch. By performing the analysis directly on the manufacturing floor, adjustments can be made quickly.

Diosynth has found the NanoDrop technology to be beneficial for microvolume UV testing at the product stream (at-line) and has reported significant savings of time and at least a fivefold improvement in efficiency. Reactions can be stopped based on a “UV gate” without wasting valuable time.

As biomanufacturing methodologies continually push the threshold of product production, detection systems need to co-evolve with the process. The dynamic range of concentration measurement capability of the NanoDrop 8000 allows measurements of the protein product to be performed at strength without dilution. The integrity of the batch biomolecule is thus maintained, providing a robust and accurate assessment of the protein product in its natural state.

On the protein side, the industry has moved to high concentration therapeutics, and any time dilutions are performed, the protein product may change its molecular configuration. NanoDrop technology allows measurement of the product in its neat configuration, giving companies greater



Figure 2. The NanoDrop 8000 is used on a movable cart for UV-Vis analysis in real time in Diosynth Biotechnology’s protein-production facility.

confidence in the final drug being delivered to the patient. The ability to analyze what is truly in a sample vial without changing it gives greater assurance to the regulatory authorities that the intent of the biomolecule has not changed during testing.

Although time was the primary driver for NanoDrop technology integration on the Diosynth manufacturing floor, reducing sample waste has been another significant benefit. Microvolume testing technology minimizes the amount of material lost during quality-control assessment. During an entire process run, traditional UV testing typically requires pulling several cuvette-filled samples for analysis. Using microvolume analysis, 1–2 microliter samples can be continually pulled off the product stream with minimal loss of product.

When moving laboratory technolo-

gies down to the manufacturing floor, software becomes an important factor for simple solution integration. The user-friendly interface of NanoDrop software relieves the burden on analysts on the floor to learn complicated software, reducing the cost of having to retrain personnel.

The ability to create custom methods also allows users to specify testing parameters and tailor analysis to specific biomolecules of interest. Diosynth personnel has found NanoDrop software to be straightforward, allowing assays and terminologies to be put into SOPs on a common platform, which avoids retraining for every specific product batch.

Microvolume spectroscopy is an example of how simple tools can be implemented throughout the processing organization including R&D, manufacturing, and quality control. Microvolume spectrophotometers can be placed

on carts and moved around the facility as needed. The ability to increase the number of replicate measurements using the eight-sample spectrophotometer throughout the process ultimately leads to a higher degree of confidence that the data produced is truly representative of the whole product (*Figure 2*).

The bioprocessing industry is continually creating new production methodologies that require novel detection technologies to maximize protein production efficiency and fidelity. Microvolume UV testing at critical points throughout process workflows can be performed on the manufacturing floor in real time with minimal loss of product, providing greater overall confidence in protein products. This type of interplay, between industry methodology development and simple solution integration, represents the future of bioprocessing optimization. **GEN**