INTRODUCTION:

The Tecan M1000 Pro Microplate reader in the Biophysics Instrumentation Facility has a specialized plate for the measurement of concentration of DNA and any attached fluorophores. This plate, called the NanoQuant plate, is the plate reader analog to a 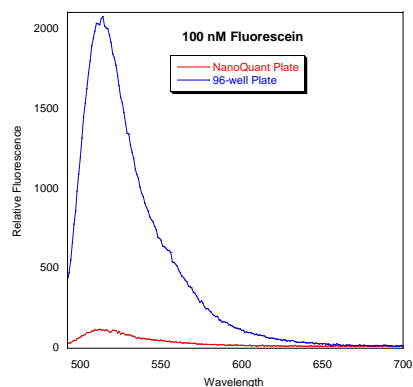NanoDrop or NanoVue system. The NanoQuant plate only requires 2 µL of sample and has a script that automatically calculates a blank subtraction and a nucleic acid concentration. The NanoQuant plate is optimized for this analysis. However, the plate should also be capable of use for a more diverse set of read types. To test this a comparison was made between a standard plate and the NanoQuant plate and its ability to measure fluorescence.

RESULTS:

To test how well the NanoQuant plate can behave like a standard 96-well plate, samples were made and then tested on both a Greiner 'U' 96-well clear plate and on the Nano-Quant plate. A dilution series of standards were prepared to compare the sensitivity of the two plates. The 96-well plate required 200 µL of solution volume, while the NanoQuant plate was loaded with 2 µL. All the wells on the NanoQuant plate were run with blank solution and then reloaded with sample and a subsequent blank subtraction was performed. For the 96-well plate, separate wells were loaded with blank solution and the samples and blanks were measured simultaneously.

Fluorescence sensitivity was tested using a fluorescein standard in 0.1 M NaOH. 96-well samples were run in triplicate and, following blank subtraction, could easily detect 1 nM fluorescein. NanoQuant samples were run in triplicate and could easily detect 10 nM fluorescein. 1 nM fluorescein was not readily detected. The fluorescence signal was about 20X greater for the 96-well plate as compared to the NanoQuant plate.

Fluorescein Concentration (nM)	Ave 96-Well (RFU)	Ave NanoQuant (RFU)
1	64.3	0.4
10	649.7	29.0
100	6414.7	298.3
1000	61769.7	3008.4
10000	OVER	30348.4
100000	OVER	OVER

CONCLUSIONS:

The NanoQuant plate is a useful tool in the measurement of absorbance and fluorescence for low volume samples. Although the plate requires about 100 times less sample the fluorescence signal was only about 20 times less. Use of a 96-well plate is faster and has higher sensitivity. In the situation where sample volume is very limited and loading times are not important, the use of the NanoQuant

plate is a viable option. See the attached method for specifics about how to use the NanoQuant plate for these measurements.

METHOD FOR THE USE OF THE NANOQUANT PLATE:

-Power up and allow M1000 to warm up for at least 15 minutes (see quick start sheet for details about plate reader startup).

-Remove the NanoQuant plate from its holder.

-Open up plate and add 2 µL of blank samples to each well. (Note: although loading each well individually is possible, it is more efficient to use a multi-channel pipet to load to all the spots at the same time to prevent evaporation.)

-Load plate into M1000.

-In i-control, under plate definition, select [NanoQuantPlate] – Tecan 16 Flat black.

-Select the wells you wish to measure (click and drag, or control-click).

-Select your measurement method.

-Click start.

-Repeat for samples and save data from Excel.