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Application Note

BioTek Synergy™ 4 Hybrid Microplate Reader

Reading the NCL 96-well Micro-Volume IMAPlate

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Nucleic acids absorb UV light at a wavelength of 260 nm. This absorption at 260 nm enables the spectroscopic quantitation of DNA or RNA which is a common routine in many laboratories. In quantitating DNA and RNA samples, the amount of available sample can be very minute. Cuvettes originally holding 3 mL are now available in 10-20 µL volumes. Although 96-well microplates can provide a 1 cm light path with 350 µL, in practice a reduced volume is used. The minimum required volume for 96-well microplates is 100 µL, for a half area 96-well plate is 50 µL and for a 384-well plate is 20-25 µL. Sample sizes below those volumes have to be diluted to at least the required minimum volume with the diluent. However, microplate readers are only sensitive to maybe 2 µg / mL of DNA and diluting a sample can lower the concentration below detectable limits.

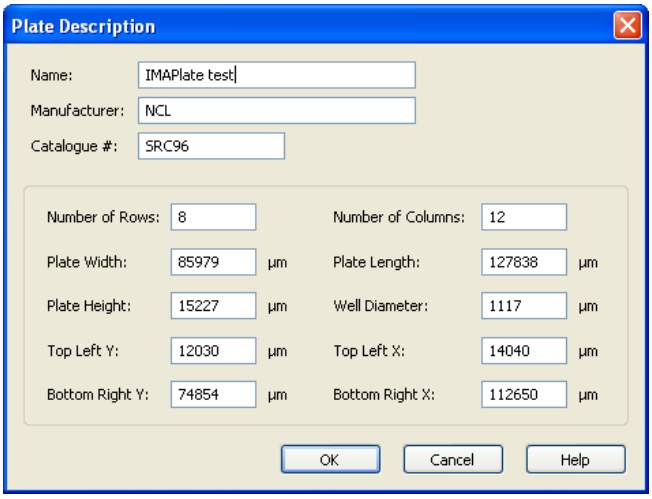
NCL New Concept Lab GmbH of Switzerland has developed the IMAPlate 5RC96, a disposable 96-well polystyrene microplate with 96 identical funnel-like reaction units. Each reaction unit contains a 5 µL round reaction chamber with a length of 5 mm that is open at the top and bottom. Once placed into an adaptor supplied with the plate, the samples can be read by a standard 96-well microplate plate reader.

The ability to accommodate small sample sizes of 1 to 5µL in a 96-well array makes this an attractive device for rapidly reading small DNA and RNA samples in a microplate format. Because the bottom is open, other applications include use as a 96-channel pipette for simultaneous liquid transfer. Sample or reagent solution can be drawn into the reaction chamber by capillary action through the bottom opening, and can remain in the reaction chamber by the adhesion and cohesive force for reaction or analysis. Solution can be discarded by using filter paper or transferred to a 96-well plate by centrifugation. In this application, we use the 96-micro cuvette array for UV spectroscopic analysis.

Materials and Methods

A stock solution of 50 µg/mL of herring sperm DNA was diluted in deionized water in tubes down to 1.7 µg/mL. A 2 to 20 µL finnpipette was used to pipette 5 µL aliquots into the IMAPlate 5RC96 black 96-well microplate for measurement at 260 nm on the Synergy 4 Hybrid Microplate Reader. Each dilution was pipetted in replicates of 8. Per the recommendation of the manufacturer, the plate was turned upside down and the 5 µL samples were pipetted into the wells through the open access in the bottom of each measurement chamber. Deionized water was similarly pipetted in 5 µL volumes into 8 wells and used as a blank. Two columns were left empty. The concentration of each aliquot was previously determined using the Synergy 4 to read samples in 200 µL microplate volumes in a Corning 3635 UV 96-well microplate. A pathlength correction algorithm was used to correct absorbances to 1 cm for calculation of concentrations and use on the standard curve.

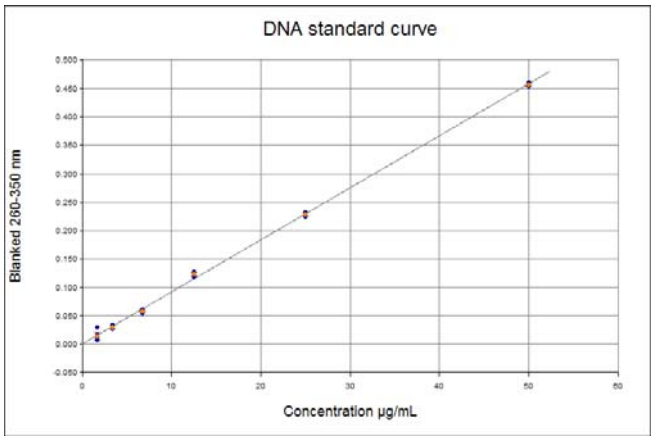
The measurements below correspond to a plate moved to the lower left corner of the adapter provided with the NCL kit.



After pipetting the standards and blank solutions (deionized water) into the plate, the plate/adapter complex was placed into the Synergy 4 and read at 260 and at 350 nm. The data of Abs260 – Abs350 is used for calculation and plotting.

Results

Standard curve concentrations are very linear over the entire range.



5 µL of herring sperm DNA in concentrations ranging from 1.7 ug/mL to 50 ug/mL in replicates of 8 was pipetted into a NCL 96-well IMAPlate and read at 260 nm and 350 nm in a Synergy 4 Hybrid Microplate Reader and (Abs260 – Abs350) is used for this plotting. All data was blanked against an average of 8 wells of deionized water.

Raw Data

The raw 260 nm, 350 nm and derived 260 nm – 350 nm data shows the corrected absorbances for the blank wells in red, and various concentrations in blue. Note that two wells, E7 and F7 were masked because they were judged to be flyers based on the 350 nm measurement.

	Blank	STD1	STD2	STD3	STD4	STD5	STD6
	1	2	3	4	5	6	7
A	0.006	0.011	0.061	0.035	0.126	0.227	0.453
B	0.001	0.017	0.061	0.031	0.121	0.228	0.456
C	-0.005	0.03	0.062	0.029	0.122	0.233	0.457
D	-0.005	0.012	0.055	0.028	0.125	0.231	0.461
E	0.001	0.013	0.059	0.03	0.124	0.225	*0.718*
F	0.003	0.014	0.057	0.029	0.128	0.232	*0.783*
G	-0.008	0.01	0.059	0.03	0.118	0.225	0.458
H	0.005	0.007	0.059	0.027	0.125	0.229	0.458

Variability in the Data

The mean, standard deviation and %CV for the standards were calculated.

Well ID	Concentration (µg/mL)	Blanked 260-350 - Mean	Std Dev	CV (%)
STD1	1.7	0.014	0.007	48.3
STD2	3.4	0.03	0.002	7.8
STD3	6.7	0.059	0.002	3.8
STD4	12.5	0.124	0.003	2.6
STD5	25	0.229	0.003	1.4
STD6	50	0.457	0.003	0.6

Discussion

When working with very low sample volumes, small assay interferences (e.g. bubbles, particles, homogeneity of solution) can have a large impact on the results. Therefore, reverse pipetting is recommended to avoid forming bubbles or incomplete dispensing from the tip. Also, it is important to use a reference measurement of the baseline, namely at 350 nm when using the IMAPlate

Precision in replicate data was <10% CV at concentrations above 3 µg/mL and < 5% above 6 µg/mL. This is suitable for quantification of small volume DNA samples.

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