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## **NanoQuant Plate™**

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### Quick Guide

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# **1. General Information**

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## **1.1 Introduction**

Tecan's NanoQuant Plate is intended as a general laboratory measurement tool for the quantification of small volumes (2µl) of nucleic acids in absorbance mode and the measurement of the labeling efficiency of nucleic acids labeled with fluorescent dyes. The NanoQuant Plate permits the application and parallel measurement of 16 different samples in a single measurement procedure. After the measurement, which is controlled by Tecan's i-control software, the calculation of nucleic acid content and purity check using the 260/280 ratio is performed automatically and the results are displayed in an Excel sheet. A blanking measurement, including an integrated reference wavelength at the beginning of the measurement procedure, functions simultaneously as a quality control check for the entire plate and indicates any pipetting or cleaning mistakes. The plate has been designed to meet the requirements of research laboratories working with various types of low-volume samples including fluorophore-labeled nucleotides.

## **1.2 Contents of the NanoQuant Plate Package**

The NanoQuant Plate package for Infinite reader series contains the following items:

- NanoQuant Plate
- Pipetting Aid
- Safety Certificate
- This Quick Guide
- Storage Box

The NanoQuant Plate is available for the following readers:

- Infinite M200 PRO
- Infinite F200 PRO
- Infinite M200 PRO NanoQuant
- Infinite F200 PRO NanoQuant
- Infinite M1000
- Infinite F500

## 1.3 Computer Requirements

The following computer requirements are needed to use the i-control software:

	Minimum	Recommended
PC	<u>Windows XP/Vista (32 bit)/Windows 7 (32 or 64 bit)</u> Windows compatible PC with a Pentium compatible processor running at 1 GHz	2 GHz (Dual Core)
Operating System	Windows XP (32-bit) SP3 Windows Vista (32-bit) Windows 7 (32-bit) Windows 7 (64-bit)	Windows XP (32-bit) SP3
Memory	<u>Windows XP</u> : 512 MB RAM <u>Windows Vista (32-bit)</u> : 1 GB RAM <u>Windows 7 (32-bit)</u> : 1 GB RAM <u>Windows 7 (64-bit)</u> : 2 GB RAM	1 GB RAM 2 GB RAM 2 GB RAM 3 GB RAM
Space Requirements	700 MB	1 GB
Monitor	Super VGA Graphics	
Resolution	1024 x 768	1280 x 1024
Color Depth	256	
Mouse	Microsoft mouse or compatible pointing device	
Communication	1 x USB 2.0	2 x USB 2.0, 1 x RS232 (Serial)
Devices	1 x CD-ROM drive <u>Windows Vista</u> : DirectX 9 graphics and 32 MB of graphics memory (for Home Basic); 128 MB of graphics memory plus WDDM support for all other versions <u>Windows 7</u> : DirectX 9 graphics device with WDDM 1.0 or higher driver	
.NET	Microsoft .NET Framework 2.0 If this version is not present, the install/upgrade program will install it side-by-side with any existing installations of the .NET Framework.	
Windows Installer	3.1 If this version is not present, the install/upgrade program will install it.	
Microsoft Excel	2002, 2003, 2007, 2010 (32-bit) – Starter edition NOT supported!	

## 1.4 System Requirements

To perform a NanoQuant measurement, the following items are required:

### Infinite 200 Pro

- An Infinite 200 Pro reader with firmware version V2.0 or higher
- A computer with i-control V1.4 Service Pack 1 or higher installed
- The NanoQuant Plate Package

Make sure the following absorbance filters are available on the filter slide of your Infinite F200 PRO:

- Position 1: 260 nm (5 nm bandwidth)
- Position 2: 280 nm (3 nm bandwidth)
- Position 3: 340 nm (10 nm bandwidth)
- Position 4: free (for individual use)

For Infinite F200 PRO, the filter positions must remain in the order in which they were delivered. The original filter positions guarantee the fastest filter switching for well-wise measurements. The Infinite M200 PRO can be used immediately for measurement without any calibration of the monochromator.

### Infinite M1000, Infinite F500

- An Infinite M1000 reader (REF 30061442) with main firmware version V2.0 or higher
- An Infinite F500
- Software: i-control V1.8 or higher
- The NanoQuant Plate Package

Make sure the following absorbance filters are available on the filter slide of your Infinite F500:

- Position 1: 260 nm (5 nm bandwidth)
- Position 2: 280 nm (3 nm bandwidth)
- Position 3: 340 nm (10 nm bandwidth)

### **Note**

Only use the Infinite reader and the NanoQuant Plate at room temperature and under normal laboratory conditions.

## **1.5 Applications**

### **Nucleic Acid Quantification (Infinite 200 PRO, Infinite M1000, Infinite F500)**

For the quantification procedure in the NanoQuant Plate, a sample volume of 2 µl is sufficient for accurate results. Absorbance of nucleic acid samples is measured at 260 nm. The optical path length of the NanoQuant Plate is 0.5 mm. To assess the purity of the nucleic acid, an additional measurement at 280 nm is performed to indicate proteins in the sample. For pure nucleic acids, a 260/280 ratio between 1.8 - 1.9 is acceptable. If this ratio is lower than 1.8 it may indicate the presence of proteins or other contaminants. If this is the case, an additional purification step/procedure is recommended.

### **Labeling Efficiency (Infinite M200 PRO and Infinite M1000 only)**

Working with nucleic acids labeled with fluorescent dyes requires samples of high quality. Besides common nucleic acid quantification and nucleic acid purity check with 260/280, the labeling efficiency is an important criterion for improved research results. With the NanoQuant Plate it is possible to measure absorbance of nucleic acids labeled with Cy3, Cy5, Alexa 555, Alexa 647 and many other fluorescent dyes.

### **Note**

All measurements on Infinite M1000 involving NanoQuant Applications are performed using one measurement channel only.

## 2. Measurement Procedure

### 2.1 Software Installation Procedure

The i-control software is installed using the following procedure:

1. Insert the i-control software CD ROM into your CD ROM drive.
2. A window opens with different selectable options.
3. Choose Software and install i-control.
4. Follow the instructions of the Wise Installation Wizard.
5. When installation is successful, exit the Installation window.
6. Switch on and connect to Infinite instrument.

#### Note

i-control is delivered with the Infinite reader series.

### 2.2 Performing a Measurement

For applications using the NanoQuant Plate, a tab called *Applications* is implemented in i-control software, so that all measurements can be performed quickly and easily.

1. Start i-control.
2. Connect to the Infinite instrument. The standard i-control window opens.
3. Select Applications in the lower left part of the window.

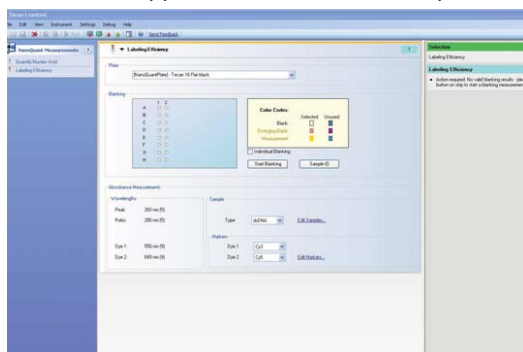


Figure 1: Overview of i-control script

4. Select the desired measurement type in the control bar on the left side of the window by double-clicking or dragging and dropping:
  - Nucleic acid quantification (Infinite 200 PRO, Infinite M1000, Infinite F500)
  - Labeling Efficiency (Infinite M200 PRO and Infinite M1000 only)
5. The corresponding measurement stripe appears and the NanoQuant Plate definition file (NanoQuant Plate Tecan 16 Flat Black) is automatically selected in the Plate field.
6. Select blanking mode. Select the **Individual Blanking** check box for individual blanking or leave the check box clear for average blanking. See Individual Blanking and Average Blanking on page 12.
7. Depending on the connected instrument, the wavelengths used for measurement are selected automatically (make sure that the correct filters are properly installed and defined on the filter slides of the Infinite F200 PRO and Infinite F500 instrument).

Instrument	Bandwidth at 260 nm	Bandwidth at 280 nm
Infinite M200 PRO:	5 nm	5 nm
Infinite F200 PRO:	5 nm	3 nm
Infinite M1000:	5 nm	5 nm
Infinite F500:	5 nm	3 nm

8. Select a sample type (e.g. dsDNA, ssDNA, RNA, etc. in the Sample type drop-down list.
9. In addition, select the respective dye(s) in the Markers drop-down lists for Labeling Efficiency measurements. If the samples are labeled with only one fluorophore, set the drop-down list of Dye 2 to None.
10. When all settings are correct, click the **Start Blanking** button to initialize the blanking measurement. The plate transport moves out and the user is requested to insert the NanoQuant Plate with the respective blanking buffer.



*Figure 2: Correct orientation of NanoQuant Plate in the reader*

11. The first measurement step is blanking with the required buffer. A separate window opens and the blanking procedure can be observed.
12. The blanking measurement is started and can be monitored in the measurement progress window. If blanking has been performed successfully, the sample positions are highlighted in yellow and the screen color changes to a homogenous green (Nucleic Acid Quantification) or blue (Labeling Efficiency). Blanking results (date and time, samples positions that were selected for blanking, blanking range, and maximum CV) are displayed next to the plate preview in the measurement stripe and saved until the instrument is disconnected.
13. When the blanking measurement has been completed successfully, the plate is moved out automatically. The plate is now ready for sample application and analysis. The green Start button is now accessible.
14. Remove any remaining blanking buffer from the sample positions by wiping the quartz spots with a piece of lint free paper and apply 2  $\mu$ l of the samples onto each spot.
15. When the NQP is loaded with samples and correctly placed onto the plate carrier, click the green Start button.

16. As the measurement is performed an Excel sheet opens automatically in the background. All measurement results (including the automatically calculated nucleic acid concentration, the 260/280 ratio, and for Labeling Efficiency measurements, the dye concentration) are concisely displayed in a matrix (analogous to the plate layout). The OD values of each sample at all relevant wavelengths are also displayed.

A		B	C	D	E	F	G	H	I	J	K
		1				2					
		diDNA									
		550 nm									
		649 nm									
		Abs				Abs					
		Value				Value					
260		0,0377	0,0	28,92	ng/µl	260		0,0362	0,0	27,77	ng/µl
280		0,0227	0,0	1,97	ratio	280		0,0218	0,0	1,99	ratio
Cy3		0,0146	0,0	1,0527	pmol/µl	Cy3		0,0140	0,0	1,0106	pmol/µl
Cy5		0,0147	0,0	0,6138	pmol/µl	Cy5		0,0141	0,0	0,6084	pmol/µl
		Abs				Abs					
		Value				Value					
260		0,0385	0,0	29,50	ng/µl	260		0,0381	0,0	29,18	ng/µl
280		0,0231	0,0	1,89	ratio	280		0,0229	0,0	2,02	ratio
Cy3		0,0149	0,0	1,0737	pmol/µl	Cy3		0,0147	0,0	1,0621	pmol/µl
Cy5		0,0150	0,0	0,6465	pmol/µl	Cy5		0,0148	0,0	0,6395	pmol/µl
		Abs				Abs					
		Value				Value					
260		0,0370	0,0	28,34	ng/µl	260		0,0355	0,0	27,19	ng/µl
280		0,0222	0,0	1,82	ratio	280		0,0213	0,0	1,83	ratio
Cy3		0,0143	0,0	1,0316	pmol/µl	Cy3		0,0137	0,0	0,9895	pmol/µl
Cy5		0,0144	0,0	0,6211	pmol/µl	Cy5		0,0138	0,0	0,5958	pmol/µl
		Abs				Abs					
		Value				Value					
260		0,0389	0,0	29,79	ng/µl	260		0,0404	0,0	30,95	ng/µl
280		0,0233	0,0	1,98	ratio	280		0,0242	0,0	1,97	ratio
Cy3		0,0150	0,0	1,0843	pmol/µl	Cy3		0,0156	0,0	1,1264	pmol/µl
Cy5		0,0151	0,0	0,6528	pmol/µl	Cy5		0,0157	0,0	0,6782	pmol/µl
		Abs				Abs					
		Value				Value					
260		0,0464	0,0	35,57	ng/µl	260		0,0386	0,0	29,59	ng/µl
280		0,0279	0,0	2,01	ratio	280		0,0232	0,0	1,92	ratio
Cy3		0,0179	0,0	1,2948	pmol/µl	Cy3		0,0149	0,0	1,0769	pmol/µl
Cy5		0,0180	0,0	0,7796	pmol/µl	Cy5		0,0150	0,0	0,6484	pmol/µl
		Abs				Abs					
		Value				Value					
260		0,0347	0,0	26,61	ng/µl	260		0,0490	0,0	37,60	ng/µl

Figure 3: Overview of Excel result sheet

17. Once the measurement procedure is finished, the plate is moved out automatically. A pop-up message appears, asking if the user wishes to perform another measurement.
- If additional (identical) measurements are to be performed, wipe off any sample residues from the previous measurement and apply new samples. Click **Yes** to start the measurement.
  - If no further measurement is to be performed, click **No**. An extra sheet appears in the Excel workbook summarizing the results of all previous measurements.

	A	B	C	D	E	F	G	H	I	J
1	dsDNA_1		260	280	Conc ng/ul	Ratio	260[raw]	280[raw]	310[raw]	Sample ID
2	A1		0.0707	0.0384	70.7	1.84	0.1201	0.0828	0.0411	Pat_1
3	A2		0.0727	0.0382	72.7	1.9	0.1184	0.0792	0.0395	Pat_2
4	B1		0.0713	0.0387	71.3	1.84	0.1239	0.0845	0.0423	Pat_3
5	B2		0.0708	0.0386	70.8	1.83	0.12	0.0823	0.0404	Pat_4
6	C1		0.0721	0.0386	72.1	1.87	0.1198	0.0816	0.0408	Pat_5
7	C2		0.0718	0.0389	71.8	1.85	0.1153	0.0778	0.0375	Pat_6
8	D1		0.0719	0.0373	71.9	1.93	0.1165	0.0782	0.0387	Pat_7
9	D2		0.0717	0.0393	71.7	1.82	0.1149	0.0776	0.0369	Pat_8
10	E1		0.073	0.0394	73	1.85	0.1201	0.0813	0.0399	Pat_9
11	E2		0.0694	0.0366	69.4	1.9	0.1193	0.0811	0.0427	Pat_10
12	F1		0.0715	0.0381	71.5	1.88	0.1174	0.0795	0.0398	Pat_11
13	F2		0.0716	0.0389	71.6	1.84	0.1183	0.0804	0.0399	Pat_12
14	G1		0.073	0.0397	73	1.84	0.1211	0.0824	0.0405	Pat_13
15	G2		0.0713	0.038	71.3	1.88	0.116	0.0783	0.0388	Pat_14
16	H1		0.0699	0.038	69.9	1.84	0.1172	0.0799	0.0389	Pat_15
17	H2		0.0683	0.037	68.3	1.85	0.1146	0.0787	0.039	Pat_16
18	dsDNA_2		260	280	Conc ng/ul	Ratio	260[raw]	280[raw]	310[raw]	Sample ID
19	A1		0.0692	0.0385	69.2	1.8	0.1195	0.0838	0.042	sample 1312
20	A2		0.0724	0.0398	72.4	1.82	0.1178	0.0805	0.0392	sample 1313
21	B1		0.0714	0.0386	71.4	1.85	0.1242	0.0846	0.0425	sample 1314
22	B2		0.0712	0.0393	71.2	1.81	0.1205	0.0831	0.0405	sample 1315
23	C1		0.072	0.0402	72	1.79	0.1191	0.0826	0.0402	sample 1316
24	C2		0.0713	0.0393	71.3	1.81	0.1151	0.0785	0.0378	sample 1317
25	D1		0.0715	0.0385	71.5	1.86	0.116	0.0793	0.0386	sample 1318
26	D2		0.0703	0.0392	70.3	1.79	0.1152	0.0792	0.0386	sample 1319
27	E1		0.0726	0.0419	72.6	1.73	0.1198	0.0839	0.04	sample 1320
28	E2		0.0699	0.0374	69.9	1.87	0.1194	0.0815	0.0423	sample 1321
29	F1		0.0715	0.0385	71.5	1.86	0.1177	0.0802	0.0401	sample 1322
30	F2		0.0718	0.0402	71.8	1.79	0.1178	0.081	0.0392	sample 1323
31	G1		0.072	0.0408	72	1.76	0.1202	0.0836	0.0406	sample 1324
32	G2		0.0706	0.0386	70.6	1.83	0.1148	0.0784	0.0383	sample 1325
33	H1		0.0676	0.0367	67.6	1.84	0.1157	0.0794	0.0397	sample 1326
34	H2		0.0686	0.0375	68.6	1.83	0.115	0.0793	0.0391	sample 1327

Figure 4: Automatic summary in Excel of all measured samples

- When all measurements are completed, clean the NanoQuant Plate thoroughly and store it appropriately.
- Once i-control has been closed, the Infinite reader can be switched off.

### Note

Excel workbooks containing the measurement results are not saved automatically. This has to be done by the user. It is possible to save the method script created in i-control for further use by clicking Save in the File menu.

## Additional blanking information

The blanking information will be stored until:

- The reader is disconnected
- The i-control is shut down
- Another sample type is selected

If other sample types are selected, the screen color will become a light green or light blue gradient again depending on the application and the blanking procedure must be repeated with corresponding wavelengths.

If you have chosen a labeling efficiency measurement before starting a blanking procedure, the type of samples and the specific wavelengths for dyes have to be selected. If the correct fluorophores are selected, the samples can be applied into the NanoQuant Plate.

## Individual Blanking and Average Blanking

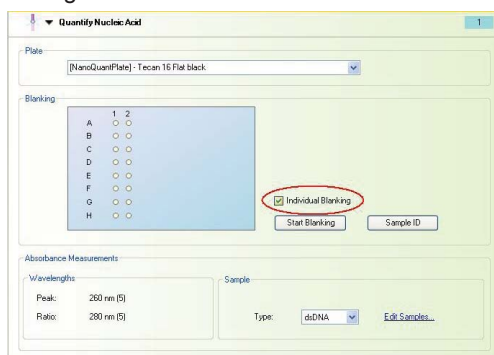
The user may choose between two distinct blanking options: Average Blanking and Individual Blanking (set by default). For Average Blanking, choose which wells are to be used for blanking by dragging a frame around the respective sample positions in the plate preview. It is generally recommended to perform the blanking measurement with all 16 sample positions, however average blanking must be done with at least two wells, in order to calculate an average value that is used to blank-correct all measured samples.

The OD results measured with average blanking have to be below 10% CV in order to grant reliable measurement results.



Figure 5: Click Start Blanking button for Average Blanking

In contrast, Individual Blanking requires blanking on all wells that are to be used for subsequent measurements. Individual blanking information is stored for each well used and blank-correction of the samples is done with the corresponding single blanking value (of the same well) instead of one average blank.



**Figure 6:** Select Individual Blanking and click the Start Blanking button to start individual blanking

It is recommended to use Individual Blanking as the standard blanking option in order to obtain the most precise and reliable results.

30	Application:	Tecan i-control	Tecan i-control , 1.8.7.0
31	Device:	infinite M1000	Serial number: 904007242
32	Firmware:	V_2_00_02/2011_S3LCE (Feb MAJ, V_2_00_02/2011_S3LCE (Feb 15 2011/11.07.57)	
33			
34	Date:	16.02.2011	
35	Time:	09:00:58	
36			
37			
38	System:	WIPEUATPC3598	
39	User:	EUTECAN\NET\supa1au	
40	Plate:	Tecan 16 Flat Bottom black Quartz/Alu [NanoQuantPlate.pdf]	
41	Plate-ID (Stacker)		
42	End Time:	09:01:44	
43			
44	Blanking:	05:53:04	
45	Time:	A1:H2	
46	Range:	260 280 310	
47			
48	A1	0.05 0.0462 0.043	
49	A2	0.05 0.0417 0.04	
50	B1	0.05 0.0472 0.044	
51	B2	0.05 0.0463 0.043	
52	C1	0.05 0.0439 0.042	
53	C2	0.04 0.0398 0.038	
54	D1	0.05 0.0418 0.04	

**Figure 7:** Individual Blanking information in the Excel result sheet

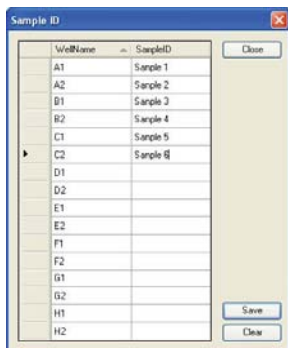
## Note

The CV value displayed in the Last Blanking box (see Figure 11) represents the variation coefficient of all wells used for blanking.

## Sample ID function

The Sample ID function allows the user to individually name each sample/well. By clicking the Sample ID box, a window opens and the user may enter the desired sample names. By clicking the Clear button, all inserted Sample IDs are deleted. Sample IDs are displayed in the summary sheet. Click **Save** to store the entries and click **Close** to complete the procedure.

Sample IDs may also be copied from Excel sheets and pasted into sample ID list.



## “Show Raw Data”

To view all measurement results as raw values, the NanoQuant tab in the Results Presentation dialog box contains the Show Raw Data check box.



By checking this box, the results summary sheet will include raw OD values at all measured wavelengths as well as blank- and reference wavelength-corrected OD values and the automatically calculated sample concentrations and ratio values.

1

	A	B	C	D	E	F	G	H	I	J
1	dsDNA_1		260	280	Conc ng/ul	Ratio	260[raw]	280[raw]	310[raw]	Sample ID
2	A1		0,071717	0,040183	71,72	*,78	0,1195	0,0856	0,0425	Sample 1
3	A2		0,071317	0,040483	71,32	*,76	0,1261	0,0929	0,0495	Sample 2
4	B1		0,068817	0,039083	68,62	*,76	0,1126	0,0809	0,0369	Sample 3
5	B2		0,068517	0,038483	68,52	*,78	0,1125	0,0801	0,0367	Sample 4
6	C1		0,068817	0,039183	68,82	*,76	0,1174	0,0854	0,0433	Sample 5
7	C2		0,067917	0,038883	67,92	*,75	0,1132	0,0818	0,04	Sample 6

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	dsDNA_Cy3_Cy5_1		260	280	Conc ng/ul	Ratio	260[raw]	280[raw]	310[raw]	Cy3	Cy5	Cy3 pmol/ul	Cy5 pmol/ul	Cy3[raw]	Cy5[raw]	Sample ID
2	A1		0,0817	0,0427	78,08	1,84	0,1253	0,0681	0,2371	0,0424	0,0003	0,0422	0,0173	0,0333	0,0327	Sample_1
3	A2		0,0754	0,0410	75,40	1,30	0,1291	0,0995	0,2382	0,0469	0,0036	0,0422	0	0,0090	0,0000	Sample_2
4	B1		0,0707	0,0391	70,98	1,41	0,1142	0,0808	0,2352	0,0387	0,0028	0,0349	0,2253	0,0319	0,0310	Sample_3
5	B2		0,0709	0,0401	70,51	1,41	0,1142	0,0809	0,2364	0,0365	0,0028	0,0402	0,2573	0,0321	0,0312	Sample_4
6	C1		0,0713	0,0388	71,87	1,71	0,1136	0,0665	0,2315	0,0435	0,0032	0	0	0,0337	0,0325	Sample_5
7	C2		0,0700	0,0403	69,80	1,40	0,1150	0,0820	0,2388	0,0462	0,0005	0,2022	0,1453	0,0323	0,0315	Sample_6

Figure 8: Raw Data Output in the Excel results summary

(1) Nucleic acid quantification

(2) Labeling efficiency

## 2.3 File menu

### i-control Toolbar



Start a measurement



Connect or disconnect an instrument



Move plate out



Move plate in



Show or hide the info pane



Open the i-control help file.

### Sample

The following sample types can be selected:

- dsDNA
- ssDNA
- RNA
- Others

Upon selecting **Others**, the **Edit Samples** window opens. The star marks a free line where additional sample types can be entered with the corresponding standard coefficient [ $\mu\text{g/ml}$ ] for 1 cm path length at 260 nm.

A ratio wavelength of 230 nm or 280 nm can also be selected from the drop-down menu.

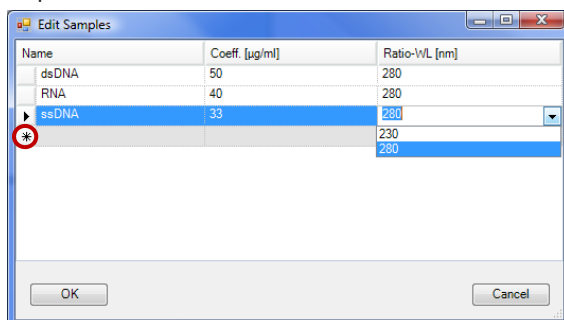


Figure 9: Selecting and adding new samples

### Note

For the Infinite F200 PRO and Infinite F500, the correct filters must always be used and defined correctly in the filter slide. If a wavelength is selected that is not available (because the filter is not in the slide) an error message appears.

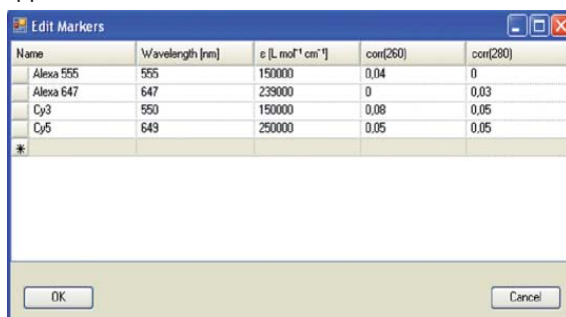


Figure 10: Editing or creating new markers

### Note

The selected sample in Edit Sample must always have the ratio wavelength set at 280 nm, otherwise no marker can be selected for Dye 1 or Dye 2 and an error message appears.

## 2.4 Quality Control of NanoQuant Plate

### Average Blanking Out of Range (CV $\geq$ 10 %)

If Average Blanking is out of range, the failed wells are highlighted in pink and an error message appears requesting the user to repeat blanking measurement.

In this case, single wells are displayed in pink, indicating these wells as diverging after blanking. These wells differ from the calculated CV by 10 %, meaning that these quartz wells are dirty due to lint, fingerprints, etc.

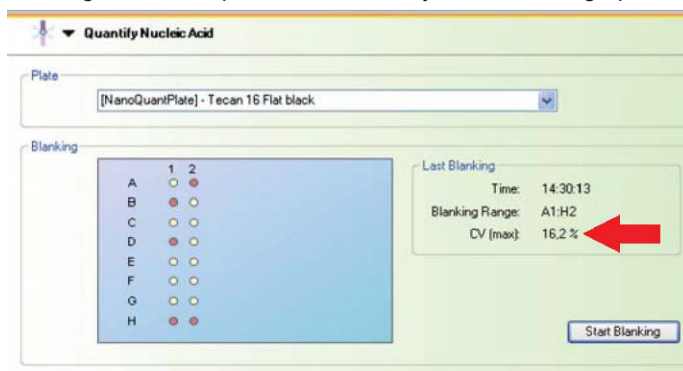
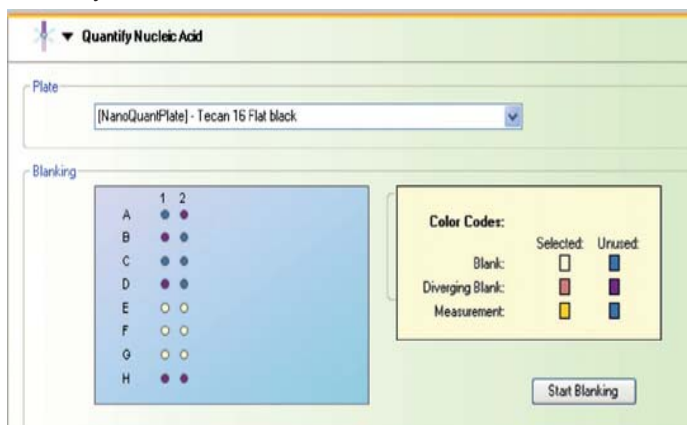


Figure 11: Color code for blanking: pink wells are out of range

There are two possibilities to complete the blanking procedure:

1. Repeat blanking with the same plate and select white wells (e.g. E1 – G2 in the example below) by dragging a frame around them. The newly selected wells will appear white, the diverging wells change from pink to purple and all other wells turn blue to indicate that they are unused.



*Figure 12: Color code for blanking procedure when re-selected: purple wells indicate formerly pink wells*

2. Move the plate out, repeat the cleaning procedure and apply fresh blanking samples into the plate and start blanking again.

## 3. NanoQuant Plate

### 3.1 Parameters

Physical	
Optics	16 quartz lenses (one per sample)
Quartz lens	Optical path length: 0.5 mm diameter: 2.2 mm
Parallel measurement	16 sample positions (2 rows of 8)
Sample volume	Min. 2 µl
Dimensions plate	Plate width: 85.4 mm Plate height: 14.6 mm Plate length: 127.8 mm Weight: ~ 160 g
Wavelength settings	
Infinite M200 PRO	260 nm (5 nm bandwidth), 280 nm (5 nm bandwidth), 310 nm (5 nm bandwidth) reference
Infinite M1000	260 nm (5 nm bandwidth), 280 nm (5 nm bandwidth), 310 nm (5 nm bandwidth) reference
Infinite F200 PRO, Infinite F500	260 nm (5 nm bandwidth), 280 nm (3 nm bandwidth), 340 nm (10 nm bandwidth) reference
Measurement time of whole plate	
Quantify Nucleic Acid	Infinite 200 PRO: 1.15 minute for 16 samples
Labeling Efficiency	Infinite 200 PRO: 2 minutes for 16 samples

Typical performance values Infinite 200 PRO	
<i>Wavelength absorbance</i>	
Wavelength accuracy	$< \pm 0.5 \text{ nm}$ for $\lambda > 315 \text{ nm}$ $< \pm 0.3 \text{ nm}$ for $\lambda \leq 315 \text{ nm}$
Wavelength reproducibility	$< \pm 0.5 \text{ nm}$ for $\lambda > 315 \text{ nm}$ $< \pm 0.3 \text{ nm}$ for $\lambda \leq 315 \text{ nm}$
Absorbance bandwidth Infinite M200 PRO	$< 5 \text{ nm}$ for $\lambda \leq 315 \text{ nm}$ $< 9 \text{ nm}$ for $\lambda > 315 \text{ nm}$
Absorbance bandwidth Infinite F200 PRO	Depending on filters used
<i>Measurement absorbance</i>	
Detection limit (DNA concentration)	1 ng/μl dsDNA
Reproducibility of one sample (50 μg/ml)	$< 1 \%$ CV
Ratio 260/280 (50 μg/ml)	$\pm 0.07$
Precision @ 260 nm	$< 0.2 \%$
Accuracy @ 260 nm	$< 0.5 \%$

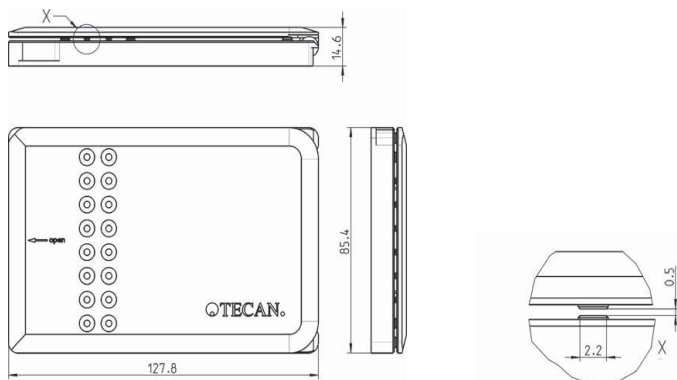


Figure 13: Technical drawing of the NanoQuant Plate (dimensions in mm)

## 3.2 Handling and Cleaning

In achieving optimal measurement results, the cleaning of the NanoQuant Plate is one of the most essential parts of the entire measurement procedure. There are two procedures for cleaning the NanoQuant Plate:

### Cleaning Procedure with Ultrasonic Bath

1. Fill an ultrasonic bath with water and place a suitable beaker filled with distilled water into the ultrasonic bath.
2. Switch on the ultrasonic and immerse the lid of the NanoQuant Plate into the beaker, with bobbing movements for about 20 seconds. Take care not to immerse the hinge of the plate.
3. Repeat the procedure with the bottom part of the NanoQuant Plate.
4. Remove any surplus water from the NanoQuant Plate with dry and oil-free compressed air.

### Cleaning Procedure with Kimwipe

1. Moisten a laboratory Kimwipe with 70% ethanol and clean the inner and outer surfaces of the NanoQuant Plate.
2. Moisten a piece of cotton or Kimwipe with distilled water and clean both sides of each quartz lens on the NanoQuant Plate.
3. Wipe off any excess liquid with a dry Kimwipe.

After cleaning, store the plate in a dirt-free and lint-free place. No lint, nor any kind of dirt or streaks, should be on the quartz lenses. Any contamination can lead to false measurements. When measuring many different samples one after the other, the quartz wells can be cleaned with a (wet) Kimwipe. The cleaning and maintenance procedures are important in order to prolong the NanoQuant Plate's lifespan and to reduce the need for servicing. It is recommended to store the cleaned NanoQuant Plate in the aluminum storage box.

#### Note

Lint, dirt or fingerprints on the quartz lenses may alter the OD values significantly! Avoid getting dirt on the spacers as well as this can lead to a change of the highly precise gap between the lid of the NanoQuant Plate and thus alter the OD values. Apply samples only onto clean quartz lenses!

### 3.3 Applying Samples

There are two different ways to apply samples on the plate:

#### Multi Channel Pipette

The fastest way to apply 16 samples onto the plate is by using an 8 fold multi channel pipette. Always use optimal tips for the multi channel pipette to ensure precise and consistent application of the samples.



*Figure 14: Applying samples onto the NanoQuant Plate*

If you need help applying the samples precisely into the wells with a multi channel pipette, use our pipetting aid: Place the pipetting aid with the indentation downward onto the quartz wells. The pipetting aid must be secured on the steel pins.



*Figure 15: NanoQuant Plate with pipetting aid*

Apply the samples into the wells. Carefully remove the pipetting aid upwards without touching the sample drops. Immediately close the lid and put the plate into the reader.

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## Single Pipette

Optionally, you can use a single pipette, but pay attention to the following:

- To increase precision and to avoid cross contamination with other samples, always use a new tip.
- Work in a timely manner – otherwise the samples may quickly evaporate, leading to false results
- Close the lid carefully and put the plate into the reader.

### Note

Always start and connect instrument before applying samples onto the plate. Work in timely manner when applying samples onto the plate to avoid evaporation of samples! Only use NanoQuant Plate at room temperature. Significant variations in temperature can lead to changes in the optical path length and therefore to imprecise OD values!

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## 3.4 NanoQuant Plate Disinfection

All parts of the NanoQuant Plate that come in contact with patient samples, positive control samples or hazardous material must be treated as potentially infectious areas. Spray or apply 70% ethanol, which is commonly used for laboratory cleaning, over the whole plate. After an exposure time of 5 minutes dry the NanoQuant Plate with a lint-free Kimwipe.

## Safety Certificate

To ensure safety and health for Tecan service personnel, our customers are kindly asked to complete the Safety Certificate (enclosed in the envelope) and sign it by a qualified person before shipping. For shipment, please attach one copy of the completed sheet to the package in which the NanoQuant Plate is returned and the other copy to the shipping documents. If a Safety Certificate is not supplied, the instrument may not be accepted by the servicing center.



## 4. Calculations

To correct the OD values due to dirt on the outer surfaces of the quartz lenses, an additional measurement at a reference wavelength is performed automatically with each measurement. For Infinite M200 PRO and Infinite M1000 readers, a reference wavelength of 310 nm is used and for the Infinite F200 PRO and Infinite F500 readers, a reference wavelength of 340 nm is used.

### 4.1 Calculation of Nucleic Acid Concentration

After the absorbance measurements of the nucleic acids have been performed in the NanoQuant Plate, i-control and Excel automatically calculate the nucleic acid concentration according to the Lambert-Beer law, including the reference values.

$$A = \epsilon \cdot d \cdot c$$

**A** Absorbance

**$\epsilon$**  Molarity Extinction Coefficient ( $\text{L mol}^{-1} \text{cm}^{-1}$ )

**d** Distance (path length in cm)

**c** Concentration ( $\text{mol L}^{-1}$ )

#### Calculation of DNA concentration:

DNA purity is automatically performed in the background as described in the following: Generally, in analytical chemistry the absorbance A is defined as

$$A_{\lambda} = \log_{10} \left( \frac{I_0}{I} \right) [\text{OD}]$$

where

**I** is the intensity of light at a specified wavelength that has passed through a sample (transmitted light intensity) and

**$I_0$**  is the intensity of the light before it enters the sample.

Absorbance measurements are often carried out in analytical chemistry, since the absorbance of a sample is proportional to the thickness of the sample and the concentration of the absorbing species in the sample. Absorbance is a logarithmic dimension; its unit is OD (Optical Density).

### **Example**

An absorbance value of 1 OD means a transmittance of 10%, and an absorbance value of 2 OD means a transmittance of 1%, etc. Because absorbance calculations are based on logarithmic dimensions, calculations between absorbance sample values and absorbance blank values are performed by subtraction and not by division. For more information review the Lambert-Beer law.

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## **4.2 Blanks**

### **Average Blanking**

The average absorbance value at 310 nm for Infinite M200 PRO and Infinite M1000 (340 nm for Infinite F200 PRO and Infinite F500) of all wells used for blanking is subtracted from the average absorbance value at 260 nm or 280 nm. The relative variation of the wells used for average blanking must be below 10% in order to be able to start a measurement.

#### **Infinite M200 PRO and Infinite M1000**

$\text{Abs}_{\text{blank average}} = \text{Abs}_{260 \text{ average}} - \text{Abs}_{310 \text{ average}} [\text{OD}]$

#### **Infinite F200 PRO and Infinite F500**

$\text{Abs}_{\text{blank average}} = \text{Abs}_{260 \text{ average}} - \text{Abs}_{340 \text{ average}} [\text{OD}]$

### **Individual Blanking**

The well-specific absorbance value at 310 nm for Infinite M200 PRO and Infinite M1000 (340 nm for Infinite F200 PRO and Infinite F500) is subtracted from the corresponding absorbance value at 260 nm.

#### **Infinite M200 PRO/Infinite M1000**

$\text{Abs}_{\text{blank A1}} = \text{Abs}_{260 \text{ A1}} - \text{Abs}_{310 \text{ A1}} [\text{OD}]$

$\text{Abs}_{\text{blank A2}} = \text{Abs}_{260 \text{ A2}} - \text{Abs}_{310 \text{ A2}} [\text{OD}]$

$\text{Abs}_{\text{blank B1}} = \text{Abs}_{260 \text{ B1}} - \text{Abs}_{310 \text{ B1}} [\text{OD}]$

etc.

### **Infinite F200 PRO/Infinite F500**

$$\text{Abs}_{\text{blank A1}} = \text{Abs}_{260 \text{ A1}} - \text{Abs}_{340 \text{ A1}} [\text{OD}]$$

$$\text{Abs}_{\text{blank A2}} = \text{Abs}_{260 \text{ A2}} - \text{Abs}_{340 \text{ A2}} [\text{OD}]$$

$$\text{Abs}_{\text{blank B1}} = \text{Abs}_{260 \text{ B1}} - \text{Abs}_{340 \text{ B1}} [\text{OD}]$$

etc.

Individual blanking information is stored for each well used and blank-correction of the samples is done with the corresponding single blanking values instead of one average blank. Every well that is to be used for sample measurement needs to be blanked beforehand.

## **4.3 Samples**

### **Calculations based on Average Blanking**

The well-specific absorbance value at 310 nm for Infinite M200 PRO and Infinite M1000 (340 nm for Infinite F200 PRO and Infinite F500) is subtracted from the corresponding absorbance value at 260 nm. Each well used for sample measurement is then blanked with the average blanking value.

### **Infinite M200 PRO/Infinite M1000**

$$\text{Abs}_{\text{A1}} = (\text{Abs}_{260 \text{ A1}} - \text{Abs}_{310 \text{ A1}}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

$$\text{Abs}_{\text{A2}} = (\text{Abs}_{260 \text{ A2}} - \text{Abs}_{310 \text{ A2}}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

$$\text{Abs}_{\text{B1}} = (\text{Abs}_{260 \text{ B1}} - \text{Abs}_{310 \text{ B1}}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

etc.

### **Infinite F200 PRO/Infinite F500**

$$\text{Abs}_{\text{A1}} = (\text{Abs}_{260 \text{ A1}} - \text{Abs}_{340 \text{ A1}}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

$$\text{Abs}_{\text{A2}} = (\text{Abs}_{260 \text{ A2}} - \text{Abs}_{340 \text{ A2}}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

$$\text{Abs}_{\text{B1}} = (\text{Abs}_{260 \text{ B1}} - \text{Abs}_{340 \text{ B1}}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

etc.

## Calculations based on Individual Blanking

The well-specific absorbance value at 310 nm for Infinite M200 PRO and Infinite M1000 (340 nm for Infinite F200 PRO and Infinite F500) is subtracted from the corresponding absorbance value at 260 nm. Each well used for sample measurement is then blanked individually with the corresponding blanking value.

### Infinite M200 PRO/Infinite M1000

$$\text{Abs}_{A1} = (\text{Abs}_{260 A1} - \text{Abs}_{310 A1}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

$$\text{Abs}_{A2} = (\text{Abs}_{260 A2} - \text{Abs}_{310 A2}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

$$\text{Abs}_{B1} = (\text{Abs}_{260 B1} - \text{Abs}_{310 B1}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

etc.

### Infinite F200 PRO/Infinite F500

$$\text{Abs}_{A1} = (\text{Abs}_{260 A1} - \text{Abs}_{340 A1}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

$$\text{Abs}_{A2} = (\text{Abs}_{260 A2} - \text{Abs}_{340 A2}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

$$\text{Abs}_{B1} = (\text{Abs}_{260 B1} - \text{Abs}_{340 B1}) - \text{Abs}_{\text{blank average}} [\text{OD}]$$

etc.

The absorbance values at 280 nm are also corrected by the corresponding absorbance values at 310 nm for Infinite M200 PRO and Infinite M1000 (or 340 nm for Infinite F200 PRO and Infinite F500). The corrected absorbance values are used for the 260/280 ratio calculation.

## 5. About the Quick Guide

This document describes the NanoQuant Plate, which has been designed as a general laboratory measurement tool to quantify small volumes (2 µl) of Nucleic Acids in absorbance mode and additionally to measure labeling efficiency of nucleic acids labeled with fluorescent dyes.

This document instructs how to:

- Perform measurement procedures using a NanoQuant Plate
- Clean and maintain the NanoQuant Plate

### Note

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