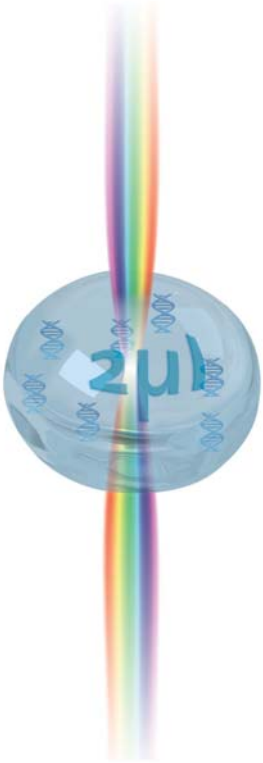
TECAN.



NanoQuant Plate™

Quick Guide

TECAN.

Table of Contents

1 General Information

1.1 Introduction

Tecan's NanoQuant Plate is intended as a general laboratory measurement tool for the quantification of small volumes (2 µl) 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 200 readers 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
- Infinite F200
- Infinite M200 NanoQuant
- Infinite F200 NanoQuant

1.3 Computer Requirements

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

Minimum	Recommended
Pentium P3 | 1.5 GHz	Pentium P4 | 2 GHz
40 GB HDD	40 GB HDD
512 MB RAM	1024 MB RAM
	2 x USB 2.0, 1 x RS232
CD ROM Drive	CD ROM Drive
Screen Resolution: 1024 x 768	Screen Resolution: 1280 x 1024
Windows XP Professional (English)	Windows XP Professional (English)
Service Pack 2	Service Pack 2
Excel 2002 (English)	Excel 2003 (English)

The i-control software is also compatible with Windows Vista (32 Bit) and Excel 2007.

1.4 System Requirements

To perform a NanoQuant measurement, the following items are required:
- An Infinite 200 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:
- 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, 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 can be used immediately for measurement without any calibration of the monochromator.

Note
Only use the Infinite 200 and the NanoQuant Plate at room temperature and under normal laboratory conditions.

1.5 Applications

Nucleic Acid Quantification (Infinite M200 and Infinite F200)

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 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.

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 the Infinite 200 instrument.

Note

i-control is delivered with the Infinite 200 reader.

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 200 instrument. The standard i-control window opens.
3. Select Applications in the lower left part of the window.

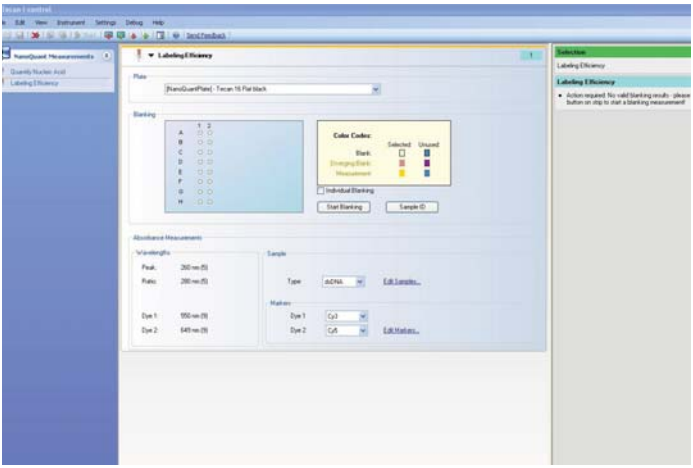


Figure 1: Overview of i-control script

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 F200 and M200)
   - *Labeling Efficiency* (Infinite M200 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 and Average Blanking on page 11.
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 filters slides of the Infinite F200 instrument).
   - **Infinite M200:**
     260 nm (5 nm bandwidth),
     280 nm (5 nm bandwidth)
   - **Infinite F200:**
     260 nm (5 nm bandwidth),
     280 nm (3 nm bandwidth)
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 µl of the samples onto each spot.
15. When the NQP is loaded with samples is 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 matrix style (analogous to the plate layout). The OD values of each sample at all relevant wavelengths are also displayed.

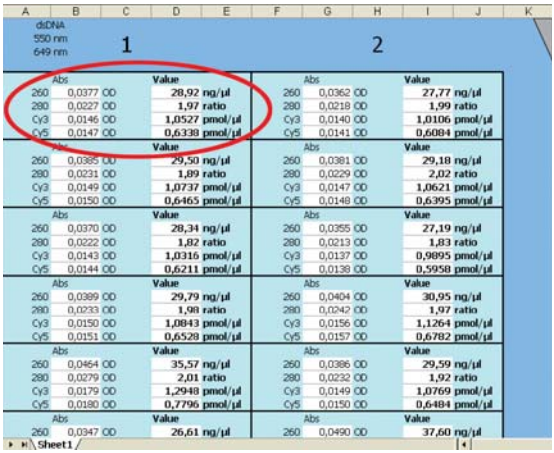
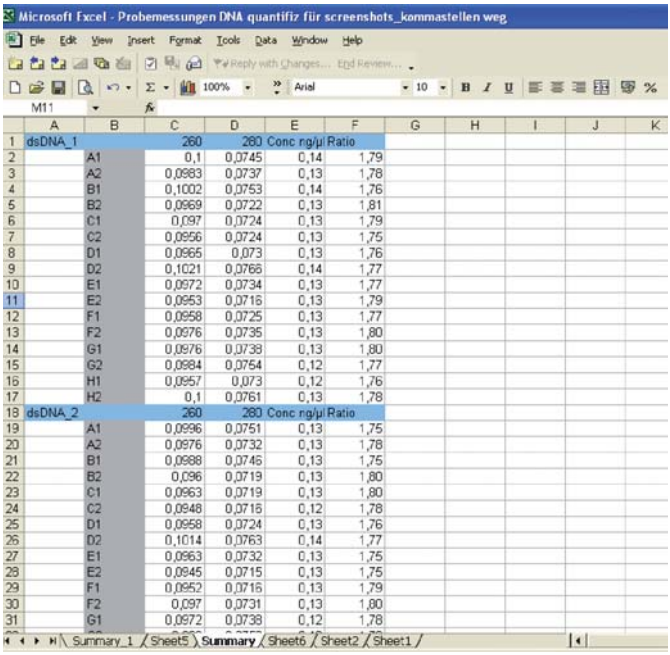


Figure 3: Overview of Excel result sheet

17. Once the measurement procedure is finished, the plate is moved out automatically. A popup 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.



Microsoft Excel - Probemessungen DNA quantifiz für screenshots_kommastellen weg

File Edit View Insert Format Tools Data Window Help

M11

	A	B	C	D	E	F	G	H	I	J	K
1	dsDNA_1		260	280	Conc ng/µl	Ratio					
2		A1	0,1	0,0745	0,14	1,79					
3		A2	0,0983	0,0737	0,13	1,78					
4		B1	0,1002	0,0753	0,14	1,76					
5		B2	0,0969	0,0722	0,13	1,81					
6		C1	0,097	0,0724	0,13	1,79					
7		C2	0,0956	0,0724	0,13	1,75					
8		D1	0,0965	0,073	0,13	1,76					
9		D2	0,1021	0,0766	0,14	1,77					
10		E1	0,0972	0,0734	0,13	1,77					
11		E2	0,0953	0,0716	0,13	1,79					
12		F1	0,0958	0,0725	0,13	1,77					
13		F2	0,0976	0,0735	0,13	1,80					
14		G1	0,0976	0,0738	0,13	1,80					
15		G2	0,0984	0,0764	0,12	1,77					
16		H1	0,0957	0,073	0,12	1,76					
17		H2	0,1	0,0761	0,13	1,78					
18	dsDNA_2		260	280	Conc ng/µl	Ratio					
19		A1	0,0996	0,0751	0,13	1,75					
20		A2	0,0976	0,0732	0,13	1,78					
21		B1	0,0988	0,0746	0,13	1,75					
22		B2	0,096	0,0719	0,13	1,80					
23		C1	0,0963	0,0719	0,13	1,80					
24		C2	0,0948	0,0715	0,12	1,78					
25		D1	0,0958	0,0724	0,13	1,76					
26		D2	0,1014	0,0763	0,14	1,77					
27		E1	0,0963	0,0732	0,13	1,75					
28		E2	0,0945	0,0715	0,13	1,75					
29		F1	0,0952	0,0716	0,13	1,79					
30		F2	0,097	0,0731	0,13	1,80					
31		G1	0,0972	0,0738	0,12	1,78					

Summary_1 / Sheet5 / Summary / Sheet6 / Sheet2 / Sheet1 /

Figure 4: Automatic summary in Excel of all measured samples

18. When all measurements are completed, clean the NanoQuant Plate thoroughly and store it appropriately.
19. Once i-control has been closed, the Infinite 200 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 (set by default) and individual blanking.

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.

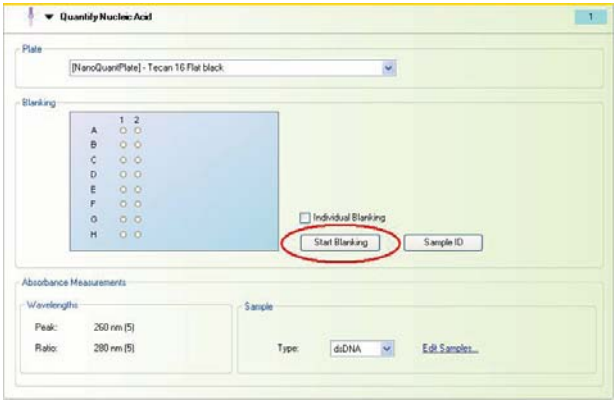


Figure 5: Click Start Blanking button for Average Blanking

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.

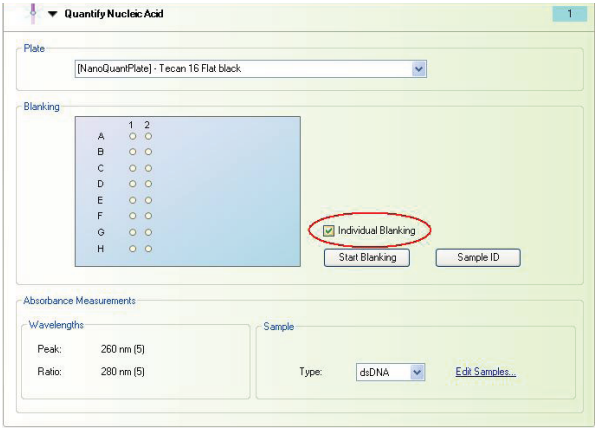


Figure 6: Select Individual Blanking and click the Start Blanking button to start individual blanking

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 , 1.6.12.0	
31	Device: infinite 200			Serial number: 609000025	Serial number of connected stacker:
32	Firmware: V_2.11_04/08_InfiniTe (Apr 4			MAI, V_2.11_04/08_InfiniTe (Apr 4 2008/14.37.11)	
33					
34	Date:	16.02.2009			
35	Time:	17:04:12			
36					
37					
38	System			WNEUATNB2697	
39	User			EUTECANNET\kafl1au	
40	Plate			Tecan 16 Flat Bottom black Quarz/Alu [NanoQuantPlate.pdfx]	
41	Plate-ID (Stacker)				
42	End Time:	17:04:47			
43					
44	Blanking:				
45	Time:	16:53:11			
46	Range:	A1:D2			
47	A1	260	0,0486	OD	
48	A2	260	0,0431	OD	
49	B1	260	0,0466	OD	
50	B2	260	0,0472	OD	
51	C1	260	0,0454	OD	
52	C2	260	0,0517	OD	

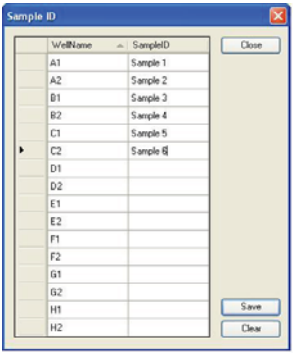
Figure 7: Individual Blanking information in the Excel result sheet

Note
The CV value displayed in the Last Blanking box (see figure 11) represents the variation coefficient of all wells used for blanking. In the case of Individual Blanking, this value is displayed only to

give the user an idea of the blanking uniformity, not for further calculations.

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.



"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/µl	Ratio	260[raw]	280[raw]	310[raw]	Sample ID
2		A1	0,071717	0,040183	71,72	1,78	0,1195	0,0856	0,0425	Sample 1
3		A2	0,071317	0,040483	71,32	1,76	0,1261	0,0929	0,0495	Sample 2
4		B1	0,068617	0,039083	68,62	1,76	0,1128	0,0809	0,0389	Sample 3
5		B2	0,068517	0,038483	68,52	1,78	0,1125	0,0801	0,0387	Sample 4
6		C1	0,068817	0,039183	68,82	1,76	0,1174	0,0854	0,0433	Sample 5
7		C2	0,067917	0,038883	67,92	1,75	0,1132	0,0818	0,04	Sample 6

2

	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/µl	Ratio	260[raw]	280[raw]	310[raw]	Cy3	Cy5	Cy3 pmol/l	Cy5 pmol/l	Cy3[raw]	Cy5[raw]	Sample ID
2		A1	0,078117	0,042783	78,08	1,83	0,1253	0,0881	0,0424	0,000317	0,000217	0,0422	0,0173	0,0333	0,0321	Sample 1
3		A2	0,075417	0,041683	75,4	1,81	0,1291	0,0935	0,0489	0,000317	-8,3E-05	0,0422	0	0,0398	0,0383	Sample 2
4		B1	0,070717	0,039183	70,37	1,81	0,1142	0,0808	0,0387	0,002617	0,002817	0,3489	0,2253	0,0319	0,031	Sample 3
5		B2	0,070917	0,039483	70,51	1,8	0,1142	0,0809	0,0385	0,003017	0,003217	0,4022	0,2573	0,0321	0,0312	Sample 4
6		C1	0,071317	0,040083	71,37	1,78	0,1196	0,0865	0,0435	-0,00038	-0,00048	0	0	0,0337	0,0325	Sample 5
7		C2	0,070017	0,038883	69,8	1,8	0,115	0,082	0,0402	0,001517	0,001817	0,2022	0,1453	0,0323	0,0315	Sample 6
8		D1	0,073417	0,040383	73,04	1,82	0,1165	0,0816	0,0383	0,002817	0,003017	0,3756	0,2413	0,0317	0,0308	Sample 7
9		D2	0,070617	0,039583	70,7	1,78	0,1196	0,0867	0,0442	-0,00058	-0,00078	0	0	0,0342	0,0329	Sample 8

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 and
- 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 extinction coefficient at 260 nm (ε [mg cm L^{-1}]). A ratio wavelength of 230 nm or 280 nm can also be selected from the drop-down menu.

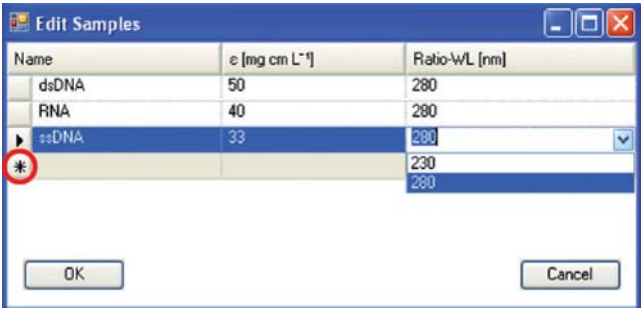


Figure 9: Selecting and adding new samples

Note
For the Infinite F200, 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.

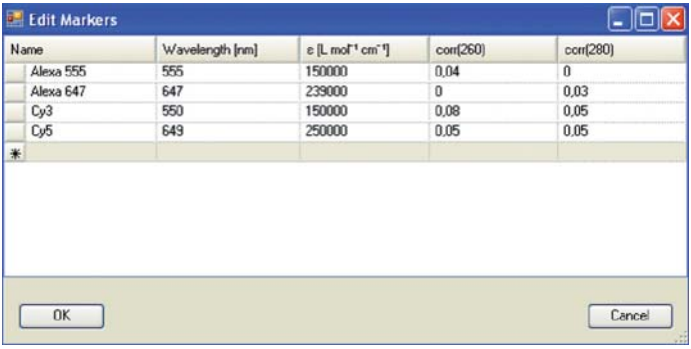


Figure 10: Editing or creating new markers

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 ≧ 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.

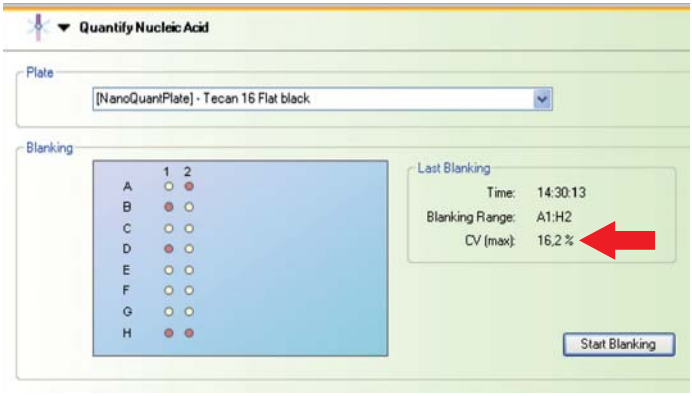


Figure 11: Color code for blanking: pink wells are out of range

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.

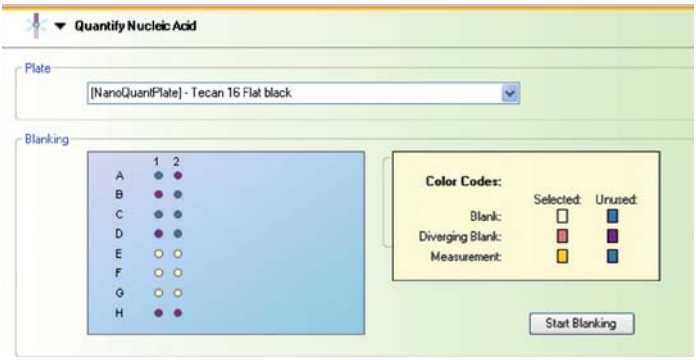


Figure 12: Color code for blanking procedure when re-selected: purple wells indicate formerly pink wells

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	260 nm (5 nm bandwidth), 280 nm (5 nm bandwidth), 310 nm (5 nm bandwidth) reference
Infinite F200	260 nm (5 nm bandwidth), 280 nm (3 nm bandwidth), 340 nm (10 nm bandwidth) reference

Measurement time of whole plate

Quantify Nucleic Acid	1.15 minute for 16 samples
Labeling Efficiency	2 minutes for 16 samples

Typical performance values

Wavelength absorbance

Wavelength accuracy	< ± 0.5 nm for λ > 315 nm < ± 0.8 nm for λ ≤ 315 nm
Wavelength reproducibility	< ± 0.5 nm for λ > 315 nm < ± 0.3 nm for λ ≤ 315 nm
Absorbance bandwidth Infinite M200	< 5 nm for λ ≤ 315 nm < 9 nm for λ > 315 nm
Absorbance bandwidth Infinite F200	Depending on filters used

Measurement absorbance

Detection limit (DNA concentration)	1 ng/µl dsDNA
Reproducibility of one sample (50 µg/ml)	< 1 % CV
Ratio 260/280 (50 µg/ml)	± 0.07
Precision @ 260 nm	< 0.2 %
Accuracy @ 260 nm	< 0.5 %

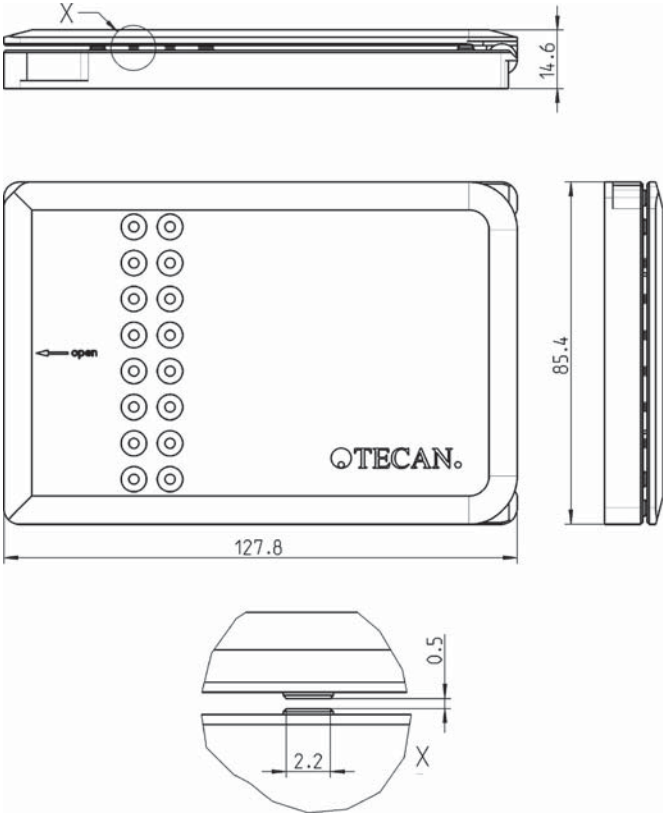


Figure 13: Technical drawing of the NanoQuant Plate (dimensions in mm)

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.

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!

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 M200 readers, a reference wavelength of 310 nm is used and for the F200 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.

$A = \varepsilon * c * d$

A *Absorbance*
ε *Extinction coefficient (l/mol $^{-cm}$)*
d *Distance – path length*
c *Concentration (molarity)*

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

$A\lambda = \log 10(I/I0)$

where I is the intensity of light at a specified wavelength λ that has passed through a sample (transmitted light intensity) and I_o 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.

Blanks

Average Blanking

The average absorbance value at 310 nm for Infinite M200 (340 nm for Infinite F200) 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

$$Abs_{blank\ average} = Abs_{260\ average} - Abs_{310\ average} \quad [OD]$$

Infinite F200

$$Abs_{blank\ average} = Abs_{260\ average} - Abs_{340\ average} \quad [OD]$$

Individual Blanking

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

Infinite M200

$$Abs_{blank\ A1} = Abs_{260\ A1} - Abs_{310\ A1} \quad [OD]$$
$$Abs_{blank\ A2} = Abs_{260\ A2} - Abs_{310\ A2} \quad [OD]$$
$$Abs_{blank\ B1} = Abs_{260\ B1} - Abs_{310\ B1} \quad [OD]$$
etc.

Infinite F200

$$Abs_{blank\ A1} = Abs_{260\ A1} - Abs_{340\ A1} \quad [OD]$$
$$Abs_{blank\ A2} = Abs_{260\ A2} - Abs_{340\ A2} \quad [OD]$$
$$Abs_{blank\ B1} = Abs_{260\ B1} - Abs_{340\ B1} \quad [OD]$$
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.

Samples

Calculations based on Average Blanking

The well-specific absorbance value at 310 nm for Infinite M200 (340 nm for Infinite F200) 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

$$Abs_{A1} = (Abs_{260\,A1} - Abs_{310\,A1}) - Abs_{blank\ average} \quad [OD]$$
$$Abs_{A2} = (Abs_{260\,A2} - Abs_{310\,A2}) - Abs_{blank\ average} \quad [OD]$$
$$Abs_{B1} = (Abs_{260\,B1} - Abs_{310\,B1}) - Abs_{blank\ average} \quad [OD]$$

Infinite F200

$$Abs_{A1} = (Abs_{260\,A1} - Abs_{340\,A1}) - Abs_{blank\ average} \quad [OD]$$
$$Abs_{A2} = (Abs_{260\,A2} - Abs_{340\,A2}) - Abs_{blank\ average} \quad [OD]$$
$$Abs_{B1} = (Abs_{260\,B1} - Abs_{340\,B1}) - Abs_{blank\ average} \quad [OD]$$

Calculations based on Individual Blanking

The well-specific absorbance value at 310 nm for Infinite M200 (340 nm for Infinite F200) 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

$$Abs_{A1} = (Abs_{260\,A1} - Abs_{310\,A1}) - Abs_{blank\ A1} \quad [OD]$$
$$Abs_{A2} = (Abs_{260\,A2} - Abs_{310\,A2}) - Abs_{blank\ A2} \quad [OD]$$
$$Abs_{B1} = (Abs_{260\,B1} - Abs_{310\,B1}) - Abs_{blank\ B1} \quad [OD]$$

etc.

Infinite F200

$$Abs_{A1} = (Abs_{260\,A1} - Abs_{340\,A1}) - Abs_{blank\ A1} \quad [OD]$$
$$Abs_{A2} = (Abs_{260\,A2} - Abs_{340\,A2}) - Abs_{blank\ A2} \quad [OD]$$
$$Abs_{B1} = (Abs_{260\,B1} - Abs_{340\,B1}) - Abs_{blank\ B1} \quad [OD]$$

etc.

The absorbance values at 280 nm are also corrected by the corresponding absorbance values at 310 nm for Infinite M200 (or 340 nm for Infinite F200). 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 (2µl) 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

Every effort has been made to avoid errors in text and diagrams; however, Tecan Austria GmbH assumes no responsibility for any errors that may appear in this publication.
It is the policy of Tecan Austria GmbH to improve products as new techniques and components become available. Tecan Austria GmbH therefore reserves the right to change specifications at any time with appropriate verification, validation, and approvals.
We would appreciate any comments on this publication.

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Warnings, Cautions and Notes

The following types of notices are used in this publication to highlight important information or to warn the user of a potentially dangerous situation:

Note: Gives helpful information.

Caution: Indicates a possibility of product damage or data loss if instructions are not followed.

Warning: Carefully read and follow the instructions provided in this document before using the software or using the product.

Warning: Directive 2002/96/EC on waste electrical and electronic equipment (WEEE): Negative enviromental impacts associated with the treatment of waste. Do not treat electrical and electronic equipment as unsorted municipal waste. collect waste electrical and electronic equipment separately.

Symbols

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