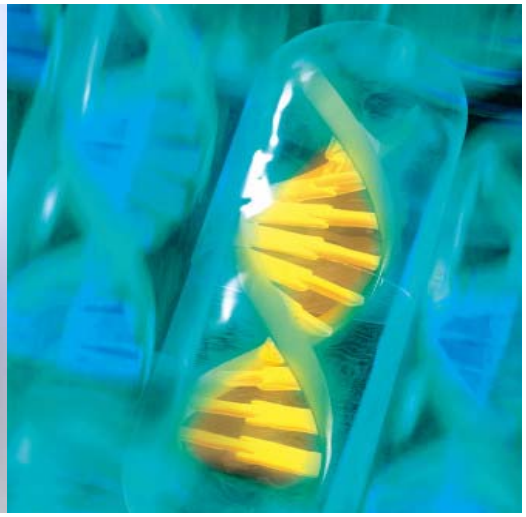
DNA and RNA quantification: fast and simple with PicoGreen® dsDNA and RiboGreen® RNA quantification reagents

Fluorescence intensity on Infinite™ F200 and Infinite M200



quad4 monochromators™

Introduction

DNA quantification

Detection and quantification of small amounts of DNA is of particular importance for a wide spectrum of biological applications, including synthesizing cDNA for generating libraries or detecting DNA impurities in drug preparations.

The most common technique for measuring nucleic acid concentration is based on measuring the absorbance at 260 nm (A_{260}), but the relative sensitivity of this method is limited to about 5 µg/ml dsDNA corresponding to an A_{260} of 0.1. Furthermore, with this measuring technique it is not possible to distinguish between RNA, ssDNA and dsDNA.

Other techniques to measure DNA use the binding of fluorescent dyes to DNA molecules. Hoechst dyes, like the bisbenzimide dye 33258, allow detection and quantification of DNA concentrations down to 10 ng/ml (1).

Another fluorescent dye binding assay uses the Quant-iT™ PicoGreen dsDNA reagent, which is at least 400 times more sensitive than the Hoechst dye based assay (2).

This note describes the use of the fluorescence microplate reader Tecan Infinite™ F200 or M200, for detection of as little as 20 pg dsDNA in a 200 µl assay volume.

RNA quantification

Modern molecular techniques like cDNA library preparation, reverse transcription PCRs and differential display PCR require the most sensitive detection of small amounts of RNA.

The detection and quantification of RNA by absorption at 260 nm hits on the same problems as with DNA - the relative contribution of proteins to the signal, free nucleotides and contaminants arising from preparation.

Compared to an OD of 0.1 at 260 nm, which corresponds to 4 µg/ml RNA in solution, the fluorescent quantification with the RiboGreen reagent enables researchers to detect as little as 1 ng/ml RNA. Using a typical fluorescence microplate reader, even 200 pg RNA can be detected in a 200 µl assay volume (3).

With the RiboGreen kit, the linear range extends from 1 ng/ml to 1 µg/ml, using two different dye concentrations. About 20 ng/ml to 1 µg/ml can be detected by the high-range assay while the low-range assay allows detection of RNA between 1 ng/ml and 50 ng/ml.

These dilutions can easily be measured with the Tecan Infinite 200 instrument series. Using the optimal gain function, RNA concentrations from 1 µg/ml to 1 ng/ml can be measured within one single dilution series and measurement sequence, which allows the smallest amounts of RNA to be detected.

Material and methods

Instruments

- Infinite F200 filter-based detection system (Tecan, Austria)
- Infinite M200 Quad4 Monochromator™ detection system (Tecan, Austria)

Microplates

- 96-well flat bottom black Polystyrol microplates (Greiner Bio-one, Germany)

Reagents and assay performance

Reagents

- Quant-iT PicoGreen quantitation reagent (Invitrogen, CA)
- RiboGreen RNA quantitation reagent and kit (Invitrogen, CA)
- 20x TE (Invitrogen, CA)
- lambda-DNA, 100 µg/ml (Invitrogen, CA)
- nuclease-free water (Fluka BioChemika, Switzerland)

Reagent preparation

Quant-iT PicoGreen

The 20x TE buffer was diluted to a 1x TE buffer with nuclease free water. The 1x TE buffer was used for diluting the Quant-iT PicoGreen reagent, the lambda-DNA, and for the assay itself. The Quant-iT PicoGreen reagent was freshly prepared in a 200-fold dilution with 1x TE buffer and stored in the dark at room temperature.

RiboGreen

Working with RNA requires RNase-free working conditions. To prevent RNase contamination, clean disposable gloves and RNase-free sterile disposable plasticware were used during handling and for all preparations. The 20x TE buffer, included in the RiboGreen RNA quantification kit, was diluted with nuclease free water to 1x TE buffer. The RiboGreen reagent was diluted depending on the assay performed; for the low-range assay, the stock solution of RiboGreen reagent was diluted 2,000-fold and, for the high-range assay, a 200-fold dilution was prepared. All solutions should be used within a few hours to prevent photodegradation.

Assay protocol

Quant-iT PicoGreen DNA quantification

A lambda-DNA dilution series was prepared as shown in table 1. From each concentration 100 µl was added per well, together with 100 µl of the aqueous working solution of Quant-iT PicoGreen to reach a final assay volume of 200 µl. For the blank, a mix of 100 µl TE buffer and 100 µl of Quant-iT PicoGreen were used. To avoid measuring faults, three replicates of each dilution were pipetted. After incubating for five minutes in the dark at room temperature, the plate was measured in the Infinite F200 and the Infinite M200 instruments.

#	DNA concentration / ml	DNA concentration / assay (200 µl)
1	1 µg	200 ng
2	500 ng	100 ng
3	250 ng	50 ng
4	50 ng	10 ng
5	10 ng	2 ng
6	2.5 ng	500 pg
7	1500 pg	300 pg
8	250 pg	50 pg
9	100 pg	20 pg
10	25 pg	5 pg
11	0 [blank]	0 [blank]

Table 1 Dilution series of lambda-DNA 1-11 in 1 x TE buffer and DNA amount per assay volume of 200 µl.

RiboGreen RNA quantification

For the quantification of high and low concentrated RNA samples (1 µg/ml to 1 ng/ml), different ribosomal standard RNA dilution series were prepared (table 2) in 1x TE. Dark green colored rows indicate high range RNA concentration where RiboGreen is diluted 200-fold, and the light green colored rows indicate RiboGreen reagent in a 2,000-fold dilution for low concentration RNA. To each 100 µl of diluted RNA, 100 µl of the respective RiboGreen reagent dilution was added corresponding to the RNA concentration range.

#	RNA concentration [ng/ml]	RNA concentration [ng/200 µl assay]
1	1000	200
2	500	100
3	100	20
4	50	10
5	25	5
6	15	3
7	10	2
8	1	0.2
9	0 [blank]	0 [blank]

Table 2: Dilution series of ribosomal standard RNA 1-9 in 1x TE buffer and RNA amount per assay volume of 200 µl.

Measurements

Measurement settings

For both DNA and RNA quantification, the same measurement parameters were chosen on both the Infinite F200 and the M200. For the Infinite F200, the optimal gain function was selected, so that the sample containing highest RNA/DNA concentration yielded the fluorescent maximum, depending on the sample.

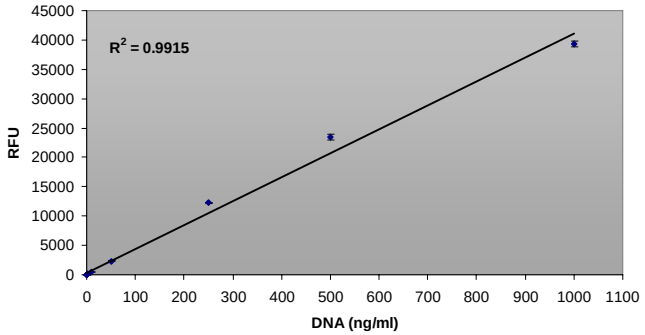
Measurement Infinite F200	
Parameter	**Setting**
Ex wavelength	485 nm
Ex bandwidth	20 nm
Em wavelength	535 nm
Em bandwidth	25 nm
Lag time	0 µs
Integration time	20 µs
Number of reads	25

Measurement Infinite M200	
Parameter	**Setting**
Ex wavelength	485 nm
Ex bandwidth	9 nm
Em wavelength	535 nm
Em bandwidth	20 nm
Lag time	0 µs
Integration time	20 µs
Number of reads	25

Table 2: Fluorescence intensity measurement parameters on Infinite F200 and on Infinite M200 with Tecan i-control™ software

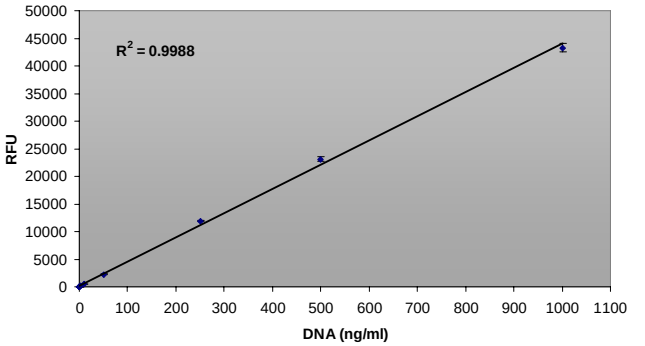
Results

DNA – Quantification with Quant-iT PicoGreen dsDNA reagent



DNA [ng/ml]	1000	250	10	1,5	0,25	0,1	0,025	0
RFU	39340	12223	464	82	15	7	2	0

Figure 1: DNA concentrations from 25 pg/ml to 1 µg/ml measured on the Infinite F200. RFUs shown calculated as triple the average minus blank.



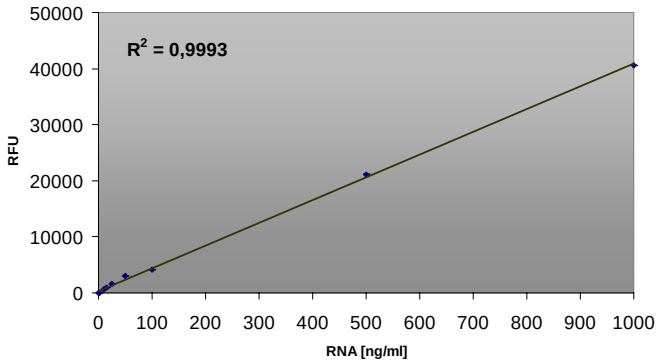
DNA [ng/ml]	1000	250	10	1,5	0,25	0,1	0,025	0
RFU	43277	11916	480	93	15	5	1	0

Figure 2: DNA concentrations from 25 pg/ml to 1 µg/ml measured on the Infinite M200. RFUs shown calculated as triple the average minus blank.

Instrument	High low range	Sensitivity in pg/ml	Sensitivity in pg/assay
Infinite F200	1000 ng/ml	100	20
Infinite M200	1000 ng/ml	100	20

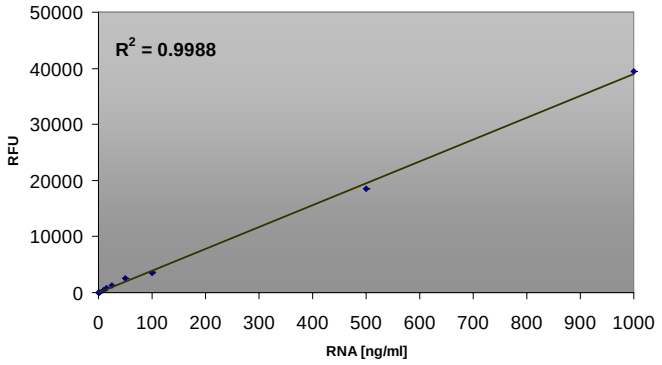
Table 3: Sensitivity of Infinite M200 and F200 instruments in high-low range measurement of DNA. Sensitivity is given in pg/ml and pg per assay volume (200 µl).

RNA – Quantification with RiboGreen reagent



RNA [ng/ml]	1000	500	100	50	25	15	10	1	0
RFU	40583	21160	4143	2964	1559	919	621	54	0

Figure 3: Measurement of high and low concentrated RNA within one dilution series on the Tecan Infinite F200. RNA was measured between 1 ng/ml and 1000 ng/ml. The table shows RFUs calculated as triple the average minus blank.



RNA [ng/ml]	1000	500	100	50	25	15	10	1	0
RFU	39429	18520	3593	2546	1339	792	530	48	0

Figure 4: Measurement of high and low concentrated RNA within one measurement on the Tecan Infinite M200. RNA was measured between 1000 ng/ml and 1 ng/ml. The table shows RFUs calculated as triple the average minus blank.

Instrument	High low range	Sensitivity in pg/ml	Sensitivity in pg/assay
Infinite F200	1000 ng/ml	150	30
Infinite M200	1000 ng/ml	200	40

Table 4: Sensitivity of Infinite M200 and F200 instruments in high-low range measurement of RNA. Sensitivity is given in pg/ml and pg per assay volume (200 µl).

Discussion

The detection and quantification of small amounts of DNA or RNA is required in many biological applications, but the most common absorbance measurement technique has the disadvantage that detection is limited to about 5 µg/ml.

For measurement and differentiation of small amounts of RNA and DNA, two detection systems using fluorescent nucleic acid binding dyes have been tested - the Quant-iT PicoGreen reagent to measure DNA and the RiboGreen reagent for detection of small amounts of RNA.

According to the manufacturer, the Quant-iT PicoGreen detection limit is 25 pg of DNA in 200 µl assay volume using a typical fluorescence microplate reader. It also recommends measuring high and low range DNA concentrations separately to obtain highly significant results.

However, using the Tecan Infinite F200 or Tecan Infinite M200 readers in combination with the Quant-iT PicoGreen kit allows the measurement of DNA easily within one single dilution series ranging from high to low DNA concentrations. In both Infinite 200 instruments the sensitivity is even better than cited and was calculated to be 20 pg DNA in 200 µl assay volume. It is also possible with these instruments to measure the smallest amounts of RNA within only one single dilution series from 1 µg/ml down to 1 ng/ml reaching a sensitivity of 30 to 40 pg RNA per assay

This advantage of the Tecan Infinite 200 series in determining DNA and RNA from high to low concentrations within one measuring sequence saves expensive working time and, most notably, valuable RNA or DNA sample material.

Conclusion

For detection and quantification of small amounts of dsDNA and RNA within a wide range of concentrations, the fluorescence intensity measurement with the Tecan Infinite 200 series provides sensitive and accurate measurement results.

List of abbreviations

FI	fluorescence intensity
ds	double stranded
ss	single stranded
RFU	Relative Fluorescence Units
20x TE	200 mM Tris-HCl, 20 mM EDTA, pH 7.5

Literature

[1] Labarca C and Paigen K: A simple, rapid, and sensitive DNA assay procedure. Analytical Biochemistry, 102, 344-352 (1980)
[2] Singer VL *et al.*: Characterization of PicoGreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation. Analytical Biochemistry, 249, 228 - 238 (1997)
[3] Jones LJ *et al.*: RNA quantitation by fluorescence-based solution assay: RiboGreen reagent characterization. Analytical Biochemistry, 265, 368 - 374 (1998)

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