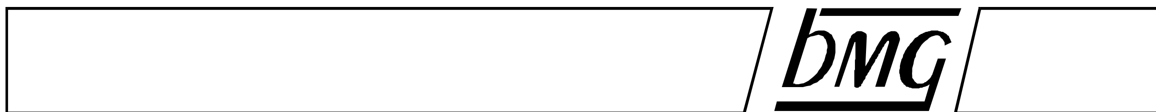

Application Note

DNA Quantification

(Absorbance Mode)

Method

The most common method for quantifying DNA samples is by conventional absorbance measurements; nucleic acids have an absorption maximum at 260nm. Most samples contain contaminates such as proteins and single stranded DNA/RNA that absorb maximally at 280nm. The equation for calculating DNA in the presence of contaminates is:

$$A_{[260]} / A_{[280]} = \text{pure dsDNA}$$

The higher the ratio, the more pure the DNA sample. It is acceptable to have a ratio between 1.8 and 2.0 for a cuvette spectrophotometer.

Comparing Results of a Spectrophometer and a Microplate Reader

Absorbance is defined by Beer-Lambert equation

$$A = \varepsilon bc$$

where ε= molar coefficient, b= pathlength and c=concentration. When the molar coefficient and pathlength are constant, absorbance is proportional to the concentration.

For a standard cuvette reader, the pathlength is usually defined as 1 centimeter. Therefore, with a conventional absorbance reading an A260 of 0.1 O.D. corresponds to 5µg/ml dsDNA solution. Because of the shorter pathlength in a microplate reader, this value is somewhat smaller (0.07 O.D. corresponds to 5µg/ml dsDNA solution).

Instrumentation

The BMG ***FLUOstar*** and ***POLARstar*** **Galaxy** are able to perform multichromatic absorbance assays. The instrument must have a low UV optical system installed (top and bottom optics).

The filters, 280-12nm and 260-12nm, should be installed on the excitation side; there should be an empty position on the emission side. Install the absorbance optic with the connection to the excitation positioning wheel and the turn the emission positioning wheel so that the bottom optics are in the 12 o’clock position.

Test Setup

Configuration

In the **Setup** menu, select **Reader Configuration** and set the instrument for absorbance. Check the **Filter** menu to be sure that the 280nm and 260nm are in the correct position.

Test Definition

Click on the test setup icon. Select the following parameters:

- Plate mode
- No. of cycles: 5
- No. of flashes: 20
- Positioning Delay: 0.5s

Plate Mode (Absorbance)

Basic Test Data | Shaking / Injection | Multichromatic

Test Name: DNA QUANT
Microplate: COSTAR 96
Layout: ABS TEST

General Settings
No. of Cycles (1...200): 5
No. of Flashes (0...200): 20
Positioning Delay (0,1...1,0s): 0.5
Horizontal Reading — Vertical Reading
Cycle Time (1...10000s): Check 120
Flying Measurement

Filter Settings
No. of Multichromatics (1...8): 2
Excitation Filter: 260abs
Emission Filter: empty
Gain (0...127): 0

Calculation
Start (1...5): 1
Stop (1...5): 1

OK | Cancel | Help

For number of multichromatics, enter ‘2’ then click on the arrow; the Multichromatics window appears.

Enter the first filter pair: **Excitation 260nm; Emission ‘empty’**
Enter the second filter pair: **Excitation 280nm; Emission ‘empty’**

Plate Mode (Absorbance)

Basic Test Data | Shaking / Injection | Multichromatic

Number	Excitation Filter	Emission Filter	Gain (0...127)
1	260abs	empty	0
2	280abs	empty	0
3			0
4			0
5			0
6			0
7			0
8			0

OK Cancel Help

Gain

Before starting the measurement, perform a gain adjustment for each filter.

Results

Open the Excel evaluation software and double click on the desired testrun. On the Raw Data page toggle the **Calc. Start and Stop** windows so that all cycles are integrated (cycles 1 to 5). In the calculation pull-down menu select ‘Average’. Press the Update button.

Open the **Evaluation96** worksheet. Setup the three tables in the following manner:

Table 1: Raw data-blank; select ‘1’ in the multichromatic box. The data from A_{260} will be shown on the table.

Table 2: Raw data-blank; select ‘2’ in the multichromatic box and the data from A_{280} will be shown.

Table 3: Select Raw data-blank. In the pull-down menu on the right, select Table1/Table2. This is the ratio A_{260}/A_{280}.

The following data was generated using a 96-well Costar low UV plate filled with 300µl solution. The DNA source was lambda phage DNA from Molecular Probes (100µg/ml). The DNA underwent a 2-fold serial dilution using a starting concentration of 50µg/ml. The dilution buffer was 1xTE.

Evaluation Type: Average

Table 1 — 1 — Raw Data - Blank

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	0.596278	0.322444	0.179178	0.097444	0.065844	0.034644	0.022511	0.018978	0.012144	0.016144	0.015944	0.011011
C												
D												
E												
F												
G												
H												

Table 2 — 2 — Raw Data - Blank

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	0.353967	0.186033	0.108867	0.058767	0.044767	0.0239	0.013433	0.015467	0.009733	0.014833	0.012767	0.007567
C												
D												
E												
F												
G												
H												

Table 1 / Table 2

Responsible for Standard Curve: — Raw Data - Blank

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	1.684559	1.733262	1.645846	1.658158	1.470836	1.449558	1.675765	1.227011	1.247717	1.08839	1.248912	1.455213
C												
D												
E												
F												
G												
H												

The acceptable ratio (in a microplate reader) for dsDNA is in the range of 1.8 to 1.4. A ratio of 1 implies that the sample is not pure enough.