Effective DNA Absorption Range of SpectroArt 200 by Using Semi-micro and Micro Cuvettes

MATERIAL

- Calf Thymus DNA Solution 1 mg/ml (Invitrogen, Carlsbad, CA, USA)
- Starna® 700 μl quartz cuvette (Starna, England)
- Hellma® 100 μl quartz cuvette (Hellma, Müllheim, Germany)
- ddH_20 for washing and diluting.

PROCEDURE

- Turn on SpectroArt 200 for 10 minutes before start operating.
- Prepare the Calf Thymus DNA series dilution as followed:

Concentration (μg/ml)	1 mg/ml Calf Thymus DNA solution (μl)	ddH_20 (μl)
0.5	0.5	999.5
2	2	998
8	8	992
10	10	990
20	20	980
30	30	970
32	32	968
40	40	960
50	50	950
60	60	940
70	70	930
80	80	920
90	90	910
100	100	900
110	110	890
120	120	880
128	128	872
140	140	860
150	150	850

- Starna® 700 μl quartz cuvette:
  1. Pipette 700 μl ddH_20 into the blank cuvette.
  2. Press on the "*DNA/RNA*" button on the touch pad.
  3. Insert the cuvette precisely into the cuvette holder.
  4. Press on the "*Blank*" button on the touch pad to measure the blank sample.
  5. Pipette 700 μl of 0.5 μg/ml DNA solution into the sample cuvette.
  6. Replace the blank sample with diluted sample.
  7. Press the "*Sample*" button on the touch pad, and repeat measure process for 5 times.
  8. Remove the sample cuvette.
  9. Wash the sample cuvette with ddH_20 for three times.
  10. Dry the cuvette with kimwipes paper as possible.
  11. Apply the next sample with different concentration to the sample cuvette.
  12. Repeat the measurement until finish all different concentration samples.

- Hellma® 100 μl quartz cuvette:
  1. Pipette 100 μl ddH_20 into the cuvette.
  2. Press on the "*DNA/RNA*" button on the touch pad.
  3. Insert the cuvette precisely into the cuvette holder.
  4. Press on the "*Blank*" button on the touch pad to measure the blank sample.
  5. Pipette 100 μl of 0.5 μg/ml DNA solution into the sample cuvette.
  6. Replace the blank sample with diluted sample.
  7. Press the "*Sample*" button on the touch pad, and repeat measure for 5 times.
  8. Remove the sample cuvette.
  9. Wash the sample cuvette with ddH_20 for three times.
  10. Dry the cuvette with kimwipes paper as possible.
  11. Apply the next sample with different concentration to the sample cuvette.
  12. Repeat the measurement until finish all different concentration samples.

RESULTS

- Starna® 700 µl quartz cuvette:

    While detecting with the Starna® 700 µl quartz cuvette with SpectroArt 200, the absorbance of DNA solution within the concentration of 10 ~ 100 µg/ml can form a linear regression with R-square value of 0.99 as we can see in figure 1(A). However, as the concentration goes up to near 100 µg/ml, the absorbance result will get lower than what it supposes to be in the linear curve. As we can see in figure 1(B), the standard curve dramatically drop when the DNA concentration pass an hundred.

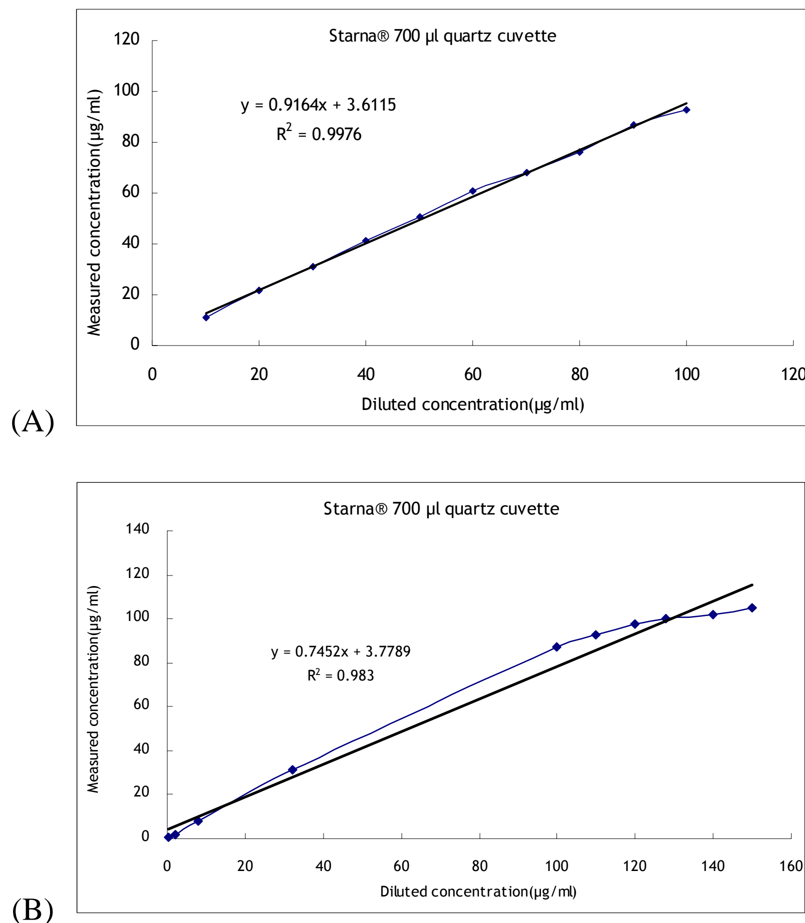

Figure 1. *Linearity of the dilution samples performed by Starna® 700 µl quartz cuvette. The X- axis is the diluted concentration from low to high. The Y-axis is the measuring value of the sample concentration. (A) Diluted concentration ranges from 10~100 µg/ml. (B) Diluted concentration ranges from 0.5~150 µg/ml.*

- Hellma® 100 μl quartz cuvette:

  Using of the Hellma® 100 μl quartz cuvette to determine the DNA concentration with SpectroArt 200, the detection result is similar to the result that detects with Starna® 700 μl quartz cuvette. As we can see in figure 2(A), the R-square value is 0.99 while the detecting concentration range is within 100 μg/ml. And as the concentration goes up to over 100 μg/ml, the absorbance result as in figure 2(B) will also get lower than what it supposes to be in the linear curve.

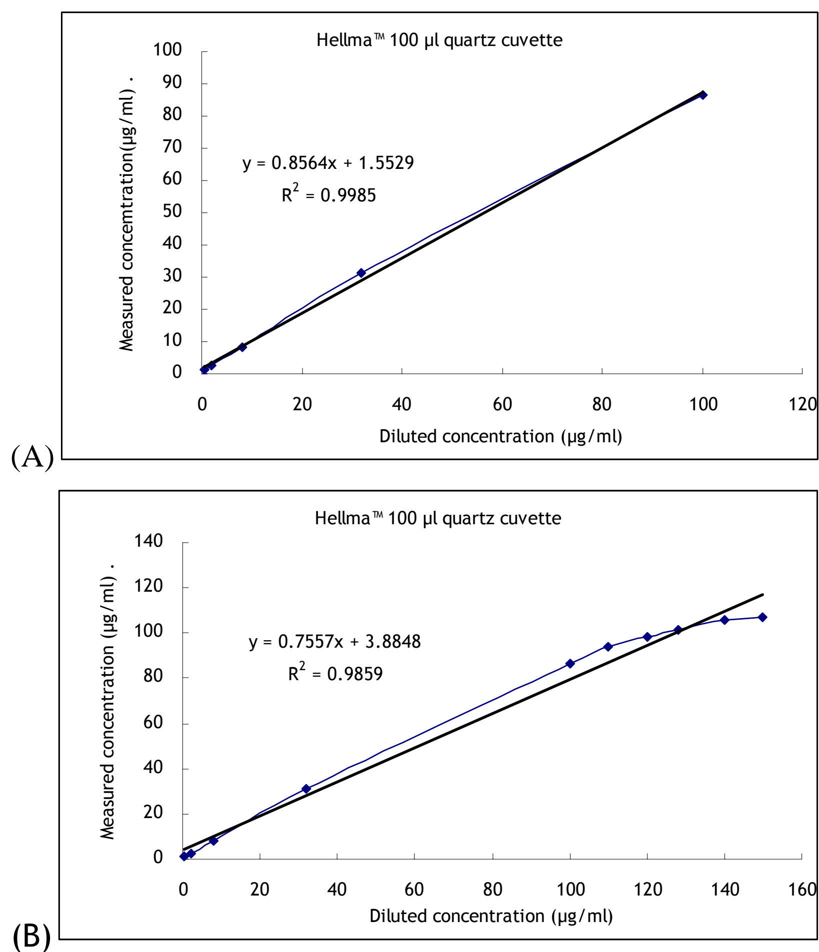

Figure 2. *Linearity of the dilution samples are performed by Hellma® 100 μl quartz cuvette. The X- axis is the diluted concentration from low to high. The Y-axis is the measuring value of the sample concentration. (A) Diluted concentration is under 100 μg/ml. (B) Diluted concentration ranges from 0.5~150 μg/ml.*

REMARKS

Measuring absorbance or transparence at any wavelength with the SpectroArt 200, the common problem that users will meet is the imprecise of the DNA concentration measurement. Most of these problems come out with the samples which have the wrong diluted concentration. According to the Beer's Law, the absorbance will increase with the rising concentration as Figure 3(A) shows below. Under this condition, absorption unit could convert to related concentration of the DNA solution. However, the measurement result will not obey the Beer's Law (Figure 3(B)) when the samples concentrations are over the effective range. This is the reason for users should notice the effective range of sample concentration.

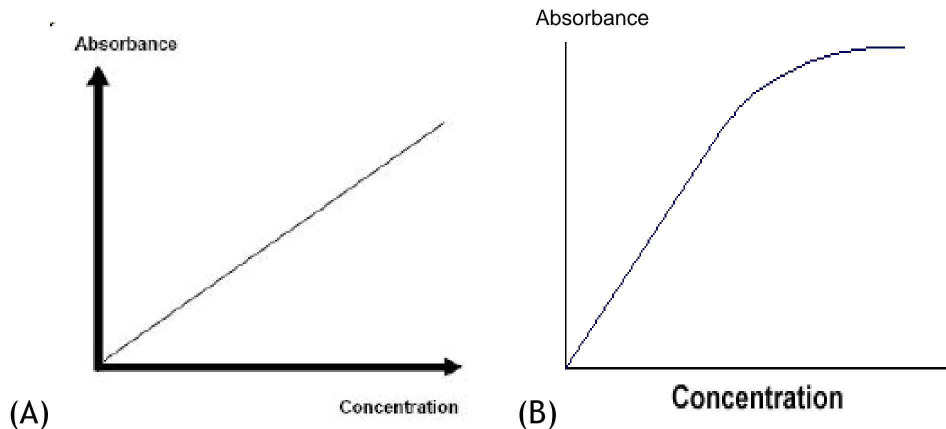

Figure 3. *Correlation of absorbance and concentration. (A) Follow the Beer's Law within the dynamic range. (B) Disobey the Beer's Law when the concentration is over the effective range.*

This Bulletin shows that when users use both different volumes cuvettes to determine the DNA concentration will have the similar effective range, because of the same dilution factor that is used to multiply. Besides, as the result shows, the DNA absorbance will dramatically drops when the concentration surpasses 100 μg/ml, and it will only form the perfect linear regression within the concentration range from 10 μg/ml to 100 μg/ml. For SpectroArt 200, the effective range of the DNA concentration detection will be located within 10 to 100 μg/ml. According to the converting factor of DNA is 50, the effective range of DNA absorbance unit will range in 0.2 to 2.0. If the absorbance unit of DNA concentration detection is out of this range, it is strongly recommended to dilute or concentrate the samples and then to repeat the measurement again to get more precise concentration result.

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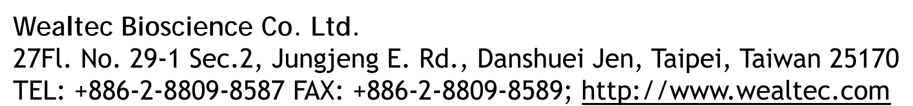
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