Nucleic Acid Detection by Ultra High Intensity Flash Xenon Lamp Spectrophotometer

INTRODUCTION

Due to the ring structure of base group, DNA and RNA both have specific absorption spectrums which can be found with significant peak at 260 nm. Basing on this feature, determination of nucleic acid concentration through spectrophotometer becomes a common method in molecular biology experiments. With the improvement on the flash Xenon lamp light source, DNA concentration determination by newly launched SpectroArt 252 spectrophotometer was tested with standard and ultra-micro volume cuvettes. Besides, the effective range of both type cuvettes was defined as well.

MATERIALS

- SpectroArt 252 (Wealtec)
- Starna® 700 µL quartz cuvette
- Hellma® TrayCell fibre-optic ultra-micro cell
- Calf Thymus DNA (Invitrogen)

PROCEDURES

1. Prepare Calf Thymus DNA into proper concentration with sterilized distilled water.
2. Sample loading:
   a. Starna® 700 µL quartz cuvette: load 700 µL DNA sample into quartz cuvette.
   b. Hellma® TrayCell: load one drop (3 µL) of DNA sample on top of the cuvette and then put on the 1.0 mm cap.
3. Measure DNA concentration with SpectroArt 252:
   a. Enter ***"DNA/RNA"*** program from manu.
   b. Set ***"dsDNA"*** as sample type and inactive "*OD320*" correction.
   c. Insert cuvette with distilled water and press ***"Blank"*** button on the touch pad.
   d. Insert cuvette with DNA sample and press ***"Sample"*** button.

e. Print out the data by pressing ***"Print"*** button.

4. Analyze the standard curve and R square value by Microsoft Office Excel software.

RESULT

(A) (B)

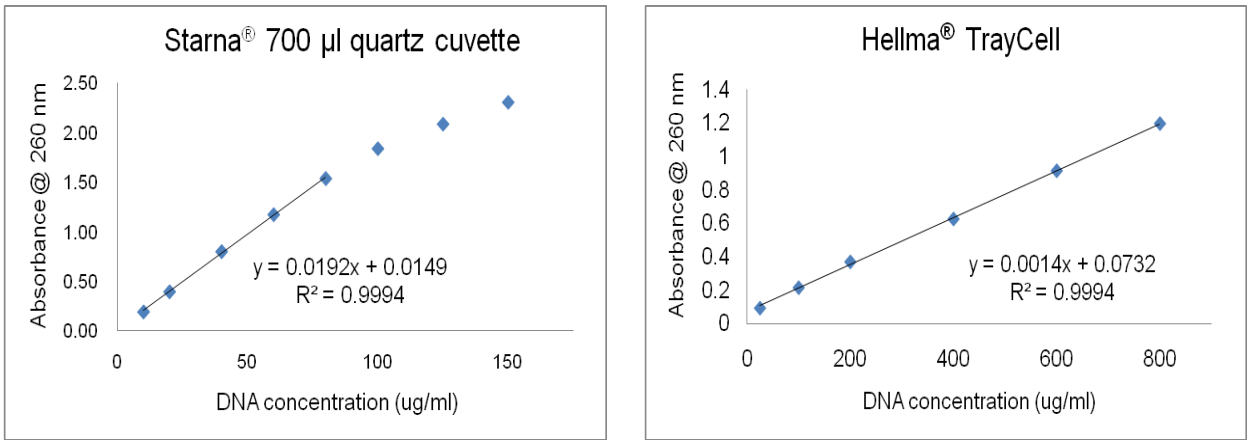

Figure 1. *The effective range of DNA measurement by using SpectroArt 252. (A) Starna® 700 µL quartz cuvette and (B) Hellma® TrayCell were used.*

DISCUSSION

According to Beer-Lambert's law, the best linear range between sample concentration and absorbance unit (AU) of spectrophotometer ranges from 0.2 to 1.2. With standard 700 µL quartz cuvette, SpectroArt 252 can measure DNA concentration from 10 (AU=0.2) to 80 (AU=1.6) µg/mL with a perfect linear correlation between AU and concentration (R square value reached 0.999) (*fig. 1A*). It means users can calculate sample amount from the given absorbance precisely within this working range. Once the absorbance unit is over the working range, the concentration will be underestimated. For instance, the linearity regression from 10 (AU=0.2) to 100 (AU=2) µg/mL sample got lower, but the R-square value was still over than 0.99.

Equipped with the high intensity flash Xenon lamp light source, SpectroArt 252 is suitable for Hellma® TrayCell which can measure samples with extremely small volume and largely lower down the needed sample volume to 0.7 ~ 5 µL. Having different reflection light paths in two sizes of caps, the dilution factors should be set with 10 for the 1.0 mm cap and with 50 for the 0.2 mm cap according to the instruction. The result of

DNA measurement by Hellma® TrayCell in SpectroArt 252 was presented as in fig. 1(B) with excellent R-square value of 0.999. Also due to use of longer reflection pathway, users should notice that any slight movement of the cell will largely affect the measurement.

As it was well known that the most common materials measured by spectrophotometer in bioscience field are nucleic acids and proteins, SpectroArt 252 was proved to have excellent performance on both kinds of samples in the present article and previous one. Equipped with high intensity flash Xenon lamp and super high sensitivity linear CCD scanning module, SpectroArt 252 provides the highest accuracy and the most reliable data on sample measurements with the easiest but completed operation interface.

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