APPLICATION NOTE

Maximizing performance of the SpectraDrop Micro-Volume Microplate for DNA quantitation

Introduction

Molecular Devices SpectraDrop™ Micro-Volume Microplate allows users to read up to 64 samples per plate on SpectraMax® Microplate Readers, with sample volumes as low as 2 µL. The SpectraDrop Microplate incorporates a specially designed adapter and a slide pair whose optical clarity allows measurements in absorbance and fluorescence modes to meet users' application needs (Figure 1). The SpectraDrop Microplate's slide design eliminates the need for calibration and gives consistent well-to-well reads with CVs below 5%.

The SpectraDrop Microplate kit consists of the following components:

- SBS-standard microplate adapter
- 24-well low volume sample slide or 64-well low volume sample slide
- 0.5 mm path length (clear spacers) cover slide for 2-µl samples
- 1.0 mm path length (blue spacers) cover slide for 4-µl samples

Measurement of nucleic acids using UV absorbance is a common method allowing quantitation of many sample types. A key consideration for optimal performance of these measurements is reduction of background noise that can result from the presence of contaminants in samples and on optical surfaces. This application note describes how to maximize performance through simple, routine care and cleaning.

Materials

- SpectraDrop Micro-Volume Microplate
- Lint-free or low-lint disposable lab wipes
- Air duster (can of compressed air)
- 70% ethanol solution (or other organic solvent such as methanol or acetone)
- Double-stranded DNA (e.g., Sigma P/N D1501-1G)
- Deionized water or TE buffer
- 8-channel pipettor capable of pipetting 2 or 4 µL
- SpectraMax Microplate Reader with absorbance detection mode

Benefits

- **Quantitate DNA down to at least 2 ng/uL**
- **Save precious sample by using 2- or 4-uL samples**
- **Measure up to 64 samples in a single plate**

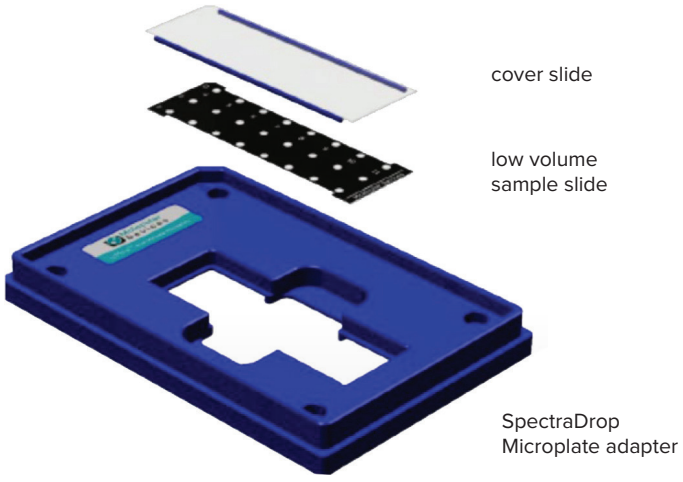

Figure 1. SpectraDrop Micro-Volume Microplate configuration. The low-volume sample slide has a mask delineating spots or 'wells' that hold 24 or 64 samples. The cover slide has 0.5-mm or 1.0-mm spacers for use with 2-µL or 4-µL samples, respectively.

Methods

Cleaning the SpectraDrop slides

Handle the SpectraDrop slides by their edges, and examine them against a dark background to identify any smudges or dust. Wearing powder-free latex or nitrile lab gloves will help to minimize fingerprints and smudging with skin oils. If smudges or dust are observed, use a disposable lab wipe to clean the slides. Remove resistant smudges or fingerprints using a water- or alcohol-dampened lab wipe. Trace-free swabs may be used in lieu of lab wipes.

For most sample measurements, simply wiping the slides with disposable lab wipes is sufficient. If sample concentrations are predicted to be low, or minimal background noise is desired, the following steps will be important. Re-examine the slides against a dark background to ensure they are free of dust particles. Remove any visible dust particles using a canned air duster to minimize background noise. Use the air duster in short, gentle bursts to blow off any dust adhering to the slides. Longer or more forceful bursts will lead to overcooling and condensation on the slides that may not be easily removed. Anti-static spray is not recommended for use with the SpectraDrop slides.

Buffers or other solutions used in measurements with the SpectraDrop Microplate can be filtered to minimize particulates that may cause elevated background values and sample-to-sample variability.

Note: Strong acids or alkali solvents should not be used with the SpectraDrop Micro-Volume Microplate because they will damage the slide mask.

Results

DNA standard curve

To demonstrate sensitivity of DNA detection with the SpectraDrop Micro-Volume Microplate, DNA standards of known concentrations ranging from 1.9 to 190 ng/µL were prepared in TE buffer and pipetted onto the wells (spots) of the SpectraDrop Micro-Volume Microplate, along with TE buffer blanks, in sets of three or six replicates using an 8-channel pipettor. Volume per spot was 2 µL using the SpectraDrop top slide with 0.5-mm spacers.

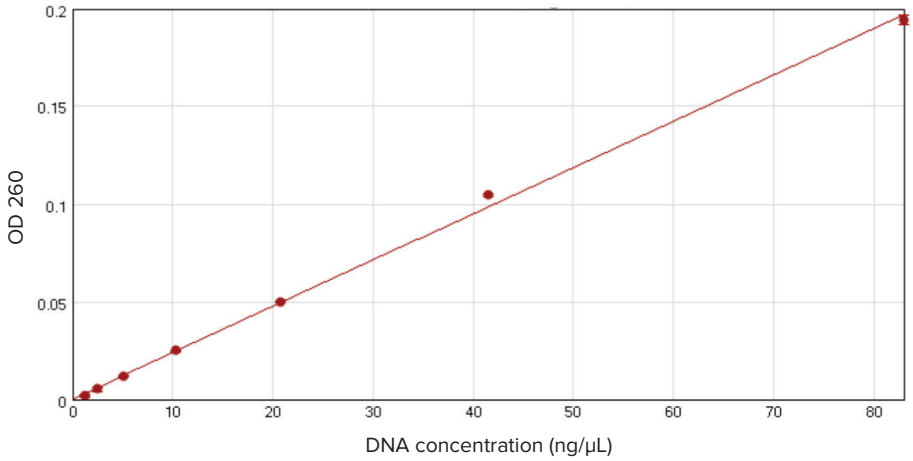

Figure 2. DNA standard curve. DNA standard curve on SpectraDrop Micro-Volume Microplate, read on a SpectraMax® M-series microplate reader. Sample slides with up to 64 wells allow users to run entire standard curves or large sample sets on a single slide.

Expt1 | Unknowns

Unknowns

Sample	Well	A260	Concentration (ng/uL)
01	E4	0.511	25.550
	E8	0.509	25.450
	F4	0.519	25.950
	F8	0.519	25.950
02	E5	0.103	5.150
	E9	0.087	4.350
	F5	0.079	3.950
	F9	0.105	5.250
03	E6	0.029	1.450
	E10	0.055	2.750
	F6	0.041	2.050
	F10	0.047	2.350

Figure 3. SoftMax Pro Software group table. Group table from SoftMax Pro Software DNA quantitation protocol for SpectraDrop Micro-Volume Microplate. Using the pathlength value for a 0.5-mm spacer slide, and concentration factor specific for double-stranded DNA in TE buffer, concentrations were calculated automatically by the software.

The SpectraDrop Plate was read on a SpectraMax M5e Microplate Reader, and data were analyzed and graphed with SoftMax® Pro Software (Figure 2). The lower limit of detection for double-stranded DNA based on a calculation of three times standard deviation of the background was less than 2 ng/µL when slides were cleaned using the guidelines above.

DNA quantitation

DNA concentration may be calculated directly from absorbance readings at 260 nm using the Beer-Lambert law, $A = x \xi c$, where A is absorbance at 260 nm, ξ is molar absorptivity, l is the length of solution through which the light passes, and c is the concentration of the solution. For double-stranded DNA dissolved in TE buffer, the concentration factor (reciprocal of molar absorptivity) is 45. The pathlength (l) for the SpectraDrop Micro-Volume Microplate is either 0.5 mm or 1.0 mm, depending on which cover slide option is used.

For SoftMax Pro software, preconfigured protocols are available to simplify the calculation of DNA concentrations for samples read using the SpectraDrop Micro-Volume Microplate. Pathlengths corresponding to the slide spacers used are applied to automatically calculate sample concentrations directly from absorbance measurements (Figure 3).

Conclusion

A key factor in maximizing performance of the SpectraDrop Micro-Volume Microplate is proper cleaning. Eliminating dust particles and smudges on the SpectraDrop Microplate's slides minimizes background absorbance and sample-to-sample variability to give optimal sensitivity and reliability of results. It is possible to exceed the sensitivity specification of 2 ng/µL when slides are cleaned carefully as described above. For many users who are measuring samples well above 2 ng/µL, simply wiping the SpectraDrop slides clean with lint-free or low-lint lab wipes is sufficient.

The SpectraDrop Micro-Volume Microplate enables sensitive measurement of small sample volumes without compromising accuracy. The SpectraDrop Microplate's slides are easy to handle, and all optical surfaces are fully accessible for ease of cleaning. No well-to-well calibration is required, as the spacer and slide design provide excellent well-to-well uniformity. 24- and 64-well sample slides, as well as cover slides with 0.5- or 1.0-mm spacers, are available to meet users' throughput and sample volume needs. The SpectraDrop Micro-Volume Microplate is compatible with all SpectraMax Readers including the SpectraMax Paradigm® Platform, as well as the StakMax® Microplate Stacker.

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