

TECHNICAL NOTE

Precision of the CyBi®-Well vario 96/60 µl Head, Example Data of Different Liquids, Pipetting Modes and Disposable Tips with Fluorescence Readout

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Key words

Precision, Fluorescence, Disposable Tips, Low Volume Handling, DMSO, Buffer, Wet and Dry Pipetting

Summary

The CyBi®-Well vario 96/60 µl head allows the parallel liquid transfer of up to 60 µl per well in one step. This volume range especially meets the requirements of medium and low volume liquid handling tasks in 96 well plates. Reformatting from 384 deep well compound storage plates into 96 well assay plates is a further typical application example.

In this study the CyBi®-Well vario 96/60 µl head was used to determine the liquid handling precision with a fluorescence readout over a broad volume range using different pipetting modes, different disposable tips and liquids with different surface tensions. The results show the excellent pipetting performance of the CyBi®-Well vario 96/60 µl head under various conditions. They support the choice of the right experimental settings and disposable tips for a broad range of customer specific applications.

Introduction

The CyBi®-Well vario is well known for fast and precise simultaneous pipetting. Nine* pipetting heads allow the reliable handling of different liquids over a broad volume range in different microplate formats. CyBio's in house specification check and quality control is performed with a standardized absorption method (p-Nitrophenol as dye solved in 0.1N NaOH) that is described in detail in every CyBi®-Well or CyBi®-Well vario manual and that is also used to determine the official specification values.

In many laboratories fluorescein solutions are used to validate liquid handling devices, because this fluorescent dye is very cost effective, stable for several months and safe to handle due to low toxicity (1). This technical note intends to complete the absorption data by fluorescence data generated with a simple fluorescence intensity measurement. However, it is important to mention, that typically the precision of absorption measurements yields a better resolution than the precision of fluorescence measurements and that simple fluorescence measurements are influenced by a bundle of parameters outside the liquid handling device that can be normalized by a multiwavelength measurement (2,3). In this technical data sheet we show precision data of the CyBi®-Well vario with the 96/60 µL head using different volumes, different tips, dye solutions with different surface tensions, and different pipetting modes that were determined by a simple fluorescence intensity measurement.

These data are example data from typical routine work in our application lab. The data also cover the low volume range below the specification limit and can only be reproduced when considering all methodical details that are described below.

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Materials and Reagents

- CyBi®-Well vario with 96/60 µl head for the handling of fluorescein working solution
 - 60 µl tips (CyBio # OL 3800-25-535-N)
 - 25 µl tips (CyBio # OL 3800-25-533-N)
 - 10 µl tips (CyBio # OL 3800-25-531-N)
- 96/250 µl head for the handling of buffer solution
 - 250µl tips (CyBio # OL 3800-25-557-N)
- 96 well plates PS black (Greiner bio-one # 655 076)
- OmniTrays (Nunc # 140156) as disposable reservoirs
- Fluorescein-Sodium (Standard Fluka # 46960)
- Fluorescein (Reference standard Molecular Probes # F1300)
- PBS (Sigma # P3813)
- DMSO (SeccoSolv Merck Darmstadt # 1.02931.1000)
- Adhesive foil (Nunc # 236269)
- PolarStar (BMG Labtechnologies) with filter set 485nm (excitation wavelength) and 520nm (emission wavelength)

Methods

The precision test was performed in black 96 well plates with a final volume of 200 µl and a final dye concentration of 300 nM. The experimental settings for the different test volumes are described in Tab.1.

| test volume [µl] | buffer volume [µl] | fluorescein working solution [µM] |
|------------------|--------------------|-----------------------------------|
| 20 | 180 | 3 |
| 10 | 190 | 6 |
| 5 | 195 | 12 |
| 2 | 198 | 30 |
| 1 | 199 | 60 |
| 0.5 | 200 | 120 |
| 0.2 | 200 | 300 |

Tab.1: Experimental settings to measure the precision of the CyBi®-Well vario 96/60 µl head

To obtain a test solution with low surface tension Fluorescein was dissolved in DMSO, for a test solution with high surface tension Fluorescein-Sodium was dissolved in PBS buffer. The working solutions with the different concentrations were prepared by diluting the dye solution with the highest concentration. All solutions were filtrated before use.

All CyBi®-Well vario pipetting methods were set up in CyBio Control 3.40 according to the following rules:

- One set of new tips per volume and pipetting mode
- Piston speed: 100 rpm (characterizes the flow rate of the liquids during aspiration/dispensing; = 8.5 µl/s with a 60 µl head at 100 rpm)
- Stage speed: 50 rpm
- Priming of tips: 1x with highest tip volume
- Break of 1s after every aspiration and dispensing step
- Immersion depth of the tips into the liquid = 1 - 2 mm
- Final volume 200 µl per well

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- Final dye concentration 300 nM
- Aspiration always with overstroke
- Pipetting back of the first cycle into the reservoir
- Pipetting of the destination volume as part of the total volume into the test plate
- Ejecting the residual volume with maximum overstroke back into the source reservoir or waste
- **Dry pipetting** means dispensing the destination volume of the dye solution about 1 mm above the bottom of the dry plate and then touching the droplet by moving the stage up, PBS buffer is used to fill the wells up to the final volume of 200 µl
- **Wet pipetting** means dispensing the desired volume with tips immersed for about 1 mm into the provided PBS buffer liquid
- Immediate sealing of the plates
- Shaking of the plates for at least 10 minutes, waiting for at least 30 minutes
- Centrifugation of the plates for 2 minutes at 2000 rpm
- Readout was performed no earlier than two hours after finishing the pipetting procedure

The precision was calculated as percentage standard deviation (coefficient of variation = CV in %) over a 384-well microplate. Three microplates were prepared per volume and the results were averaged.

Results and Discussion

In Tab.2 the precision data of the CyBi®-Well vario 96/60 µl head using different volumes, different tips, dye solutions with different surface tensions and different pipetting modes are summarized.

| Tips | Test volume | DMSO dry [%CV] | DMSO wet [%CV] | Buffer dry [%CV] | Buffer wet [%CV] |
|-------------------|-------------|----------------|----------------|------------------|------------------|
| 60 µl tips | | | | | |
| | 20 µl | 1.6 | 1.6 | 1.7 | 1.6 |
| | 10 µl | 1.7 | 1.8 | 1.8 | 1.5 |
| | 5 µl | 1.5 | 1.7 | 2.4 | 1.6 |
| | 2 µl | 1.9 | 1.5 | 3.2 | 1.8 |
| | 1 µl | 2.3 | 1.8 | 6.1 | 2.1 |
| | 0.5 µl | 3.1 | 3.2 | n. r. | 3.4 |
| | 0.2 µl | n.r. | 7.2 | n. t. | 8.8 |
| 25 µl tips | | | | | |
| | 20 µl | 1.5 | 1.9 | 1.7 | 1.5 |
| | 10 µl | 1.6 | 2.0 | 1.9 | 1.6 |
| | 5 µl | 1.7 | 1.8 | 2.5 | 1.7 |
| | 2 µl | 2.4 | 1.5 | 4.1 | 1.7 |
| | 1 µl | 2.0 | 2.3 | 7.5 | 1.6 |
| | 0.5 µl | 4.0 | 4.0 | n. r. | 2.6 |
| | 0.2 µl | n.r. | 11.9 | n. t. | 6.0 |
| 10 µl tips | | | | | |
| | 10 µl | 1.5 | 1.5 | 1.5 | 1.4 |
| | 5 µl | 1.7 | 1.6 | 1.6 | 1.5 |
| | 2 µl | 1.3 | 1.4 | 2.1 | 1.6 |
| | 1 µl | 2.3 | 2.1 | 3.6 | 2.0 |
| | 0.5 µl | 3.9 | 3.2 | n. r. | 2.8 |
| | 0.2 µl | 6.8 | 3.1 | n. t. | 4.6 |

Tab.2: Overview of precision data (fluorescence readout) that were obtained with the CyBi®-Well vario 96/60 µl head for various volumes with various tips, various liquids and various pipetting modes (n=3), constellations that result in CV values higher than 15% are not recommended (n. r.), n. t. = not tested.

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The results indicate, that the CyBi®-Well vario 96/60 µL head allows highly precise high and low volume compound and reagent handling. Fig. 1 graphically shows a summary of the precision data that were obtained with the CyBi®-Well vario 96/60 µL head and 60 µL tips with both test liquids and pipetting modes, respectively.

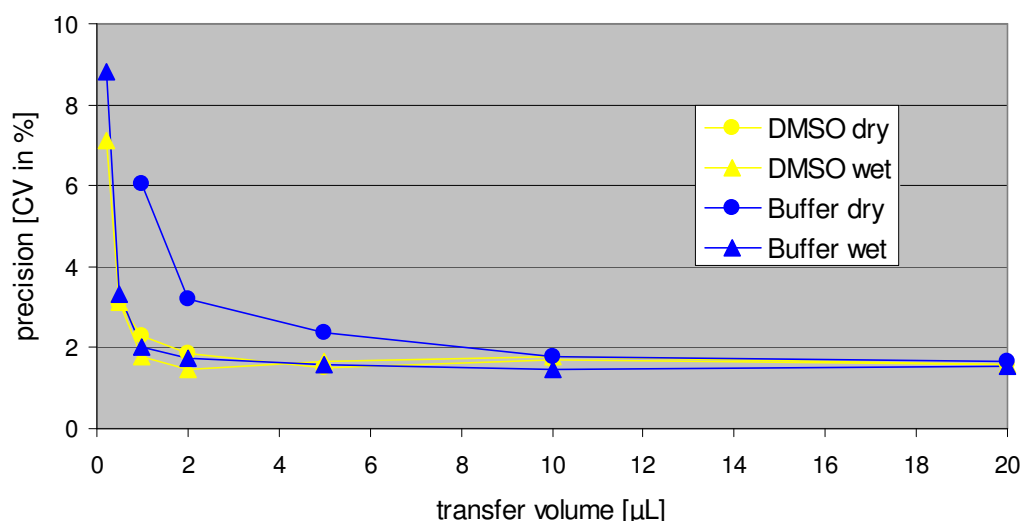


Fig. 1: Precision data (fluorescence readout) obtained with the CyBi®-Well vario 96/60 µL head, comparison of pipetting DMSO and buffer in the wet-to-dry and wet-to-wet mode with 60 µL tips, n=3.

The precision of the CyBi®-Well vario 96/60 µL head is very similar down to 1 µL with both test liquids and pipetting modes compared to the pipetting performance of the CyBi®-Well vario 96/25 µL head (4), except of dry pipetting of buffer. In the wet-to-dry pipetting mode buffer solutions still can be handled with the CyBi®-Well vario 96/60 µL head down to a volume of 1 µL if precision errors of about 6 % CV are acceptable. With further decreasing volumes the droplet formation at the tip orifice becomes more and more critical due to the high surface tension (that has to be overcome) and the precision error increases quickly to more than 15 % CV, see Tab.2). Further, the quality of the microplates, especially the flatness of the plate bottom, is essential for the outcome of wet-to-dry liquid transfers. CyBi®-Well and CyBi®-Well vario allow a vertical adjustment of the stage height, in steps of 1/10 mm, for touching the tips to the plate bottom in order to reliably deposit a droplet. Often 1/10 mm stage height difference is enough to cross the rubicon between good precision data and not acceptable results. For wet-to-wet and wet-to-dry pipetting of DMSO as well as for wet-to-wet pipetting of buffer solutions in the volume range of 0.5 µL precision errors less than 4% can be realized.

In principle, the precision of the CyBi®-Well vario 96/60 µL head is comparable to that of the CyBi®-Well vario 384/60 µL head (5). As expected, the absolute values for the precision error are slightly better for the CyBi®-Well vario 96/60 µL head.

The precision of the CyBi®-Well vario 96/60 µL head down to a volume of 1 µL is nearly comparable between 60 µL tips, 25 µL tips and 10 µL tips. For the handling of volumes lower than 0.5 µL the precision can be improved slightly by using smaller tips.

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Sometimes low volumes of compound solutions are stored in deep well plates that can not be handled with the 60 μl tips, 25 μl tips or 10 μl tips. In these cases 250 μl deep well tips of CyBio can be used also with the 96/60 μl pipetting head to prepare shallow well compound plates or to handle the compounds directly in the wet mode down to volumes as low as 1 μl (data see Tab. 3). These results are very similar to that which were obtained with the CyBi®-Well vario 96/25 μl head (4). The opportunity of the common usage of alternative disposable tips with a certain pipetting head is a general benefit of the CyBio equipment due to the special CyBio tip-sealing technology.

| Tips | Test volume [μl] | DMSO dry [%CV] | DMSO wet [%CV] |
|----------------------------------|-------------------------------|----------------|----------------|
| 250 μl deep well tips | | | |
| | 5 | 2.6 | 1.6 |
| | 2 | (n. r.) | 1.7 |
| | 1 | (n. r.) | 2.3 |

Tab.3: Precision example data (fluorescence readout) using the CyBi®-Well vario 96/60 μl head with 250 μl deep well tips for various volumes with various tips, various liquids and various pipetting modes ($n=3$), constellations that result in CV values higher than 15% are not recommended (n. r.).

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