TECHNICAL NOTE

Precision of the CyBi®-Well vario Nanoliter Head 384/2.5 µL, Example Data of Different Liquids with Fluorescence Readout

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Key words

precision, fluorescence, nanoliter handling of compounds and reagents, DMSO, buffer, wet-to-wet pipetting

Summary

In this study the CyBi®-Well vario Nanoliter Head 384/2.5 µL was used to determine the liquid handling precision with a fluorescence readout in the low volume range using buffer and DMSO in the wet-to-wet liquid transfer mode. The data shows the excellent pipetting performance of the CyBi®-Well vario Nanoliter Head 384/2.5 µL with both liquids and demonstrate the suitability for precise low volume reagent and compound handling.

Introduction

The CyBi®-Well vario is well known for fast and precise simultaneous pipetting. Six* pipetting heads allow the reliable handling of different liquids over a broad volume range.
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 (2) 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. Simple fluorescence intensity measurements are influenced by a bundle of parameters outside the liquid handling device that can be normalized by a multiwavelenght measurement (3,4).

In this technical data sheet we show precision data of the CyBi®-Well vario Nanoliter Head 384/2.5 µL using different volumes of fluorescein solutions in buffer and DMSO, respectively, that were determined by a simple fluorescence intensity measurement. The CyBi®-Well vario Nanoliter Head was especially designed for low volume wet-to-wet liquid transfer. Guidelines for method set up that cover our liquid handling experience are provided. The results are example data from typical routine work in our application lab. They also include the precision in the ultra-low volume range below the specification limit.

References:
1. Harris D.L., and Mutz M., "Debunking the Myth: Validation of Fluorescein for Testing the Precision of Nanoliter Dispensing", JALA August 2006, pp. 233-239.
2. Undisz, K., and Zimmermann, P., "Precise low volume compound handling with the new Nanoliter Head of the CyBi®-Well vario", Poster at LRIG Conference "Compound Management - Emerging Themes", 19.04.2005, London.
3. Rhode H., Schulze M., Renard S., Zimmermann P., Moore T., Cumme G. A., and Horn A., "An improved Method for Checking HTS/uHTS Liquid-Handling Systems", J Biomol Screening December 2004, 9(8), pp. 726 - 733.
4. Rhode H., Horn A., Schulze M., Cumme G., „Method for Characterizing a Highly Parallelized Liquid Handling Technique Using Microplates and Test Kit for Carrying Out the Method", W= 03/079030 A1, DE 10391021, GB 2403535

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Materials

- CyBi®-Well vario with Nanoliter Head 384/2.5 µL
  This nanoliter pipetting tool consists of the CyBi®-Well vario Nanoliter Head 384/2.5 µL and a tip magazine with 384 specifically designed ceramic tips (maximum capacity 2.5 µl, total length 41 mm, effective plate immersion depth = 26 mm). The aperture and front surface of the ceramic tips were designed to minimize the adhesion and by this way also the "pin tool effect". Innovative inlays in the upper part of the mirror-finished ceramic tips (patent pending and German Utility Model) limit the air volume and enable low volume liquid transfer with high precision. The ceramic tips are primarily designed for wet-to-wet transfer.
- CyBi®-Well vario 384/40 µL Head with 25 µL tips (CyBio # OL 2001-25-250) for buffer handling
- 384 well plates PS black (Greiner bio-one # 781 076)
- 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)
- Ethanol p.A. (VWR International # 8.18760.2500)
- 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 384 well plates with a final volume of 40 µL and a final dye concentration of 300 nM. The experimental settings for the different test volumes are described inTab.1.

test volume [µL]	buffer volume [µL]	fluorescein working solution [µM]
2	38	6
1	39	12
0.5	39.5	24
0.2	39.8	60
0.1	39.9	120
0.05	40	240
0.025	40	300

Tab.1: Experimental settings to measure the precision of the CyBi®-Well vario Nanoliter Head 384/2.5 µL

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 (300nM). All solutions were filtrated before use.

Before changing the concentration of the fluorescein working solution the tips were rinsed 5 times with deionized water containing 10% DMSO. Finally the tips were rinsed with ethanol to accelerate drying.

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

- piston speed: 80 rpm (equivalent to 4 µL/s)
- stage speed: 40 rpm
- priming of tips: 10 x with 2.5 µL
- break of 1s after every aspiration and dispensing step
- tips immersion depth 1 - 2 mm
- final volume 40 µL per well
- final dye concentration 300 nM
- aspiration always with overstroke
- dispensing back of the first pipetting cycle into the reservoir, dispensing back of two pipetting cycles for volumes below 1 µL
- pipetting of the destination volume as part of the total volume into the test plate
- always wet pipetting (dispensing the destination volume with tips immersed for about 1 mm into the provided PBS buffer liquid)
- ejecting the residual volume with immersed tips and maximum overstroke back into the source reservoir or waste
- 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 results of the CyBi®-Well vario Nanoliter Head 384/2.5 µL using different volumes and fluorescein solutions with different surface tensions are summarized.

Tips	Test volume	DMSO wet [% CV]	Buffer wet [% CV]
2.5 µL ceramic tips (41 mm)			
	2 µL	1.9	2.5
	1 µL	1.6	2.5
	0.5 µL	2.2	2.1
	0.2 µL	2.7	3.1
	0.1 µL	3.6	4.3
	0.05 µL	8.2	10.0
	0.025 µL	13.7	n. r.

Tab.2: Overview of precision results (fluorescence readout) that were obtained with the CyBi®-Well vario Nanoliter Head 384/2.5 µL for various volumes with various fluorescein solutions in the wet-to-wet pipetting mode (n=3), constellations that result in CV values higher than 15% are not recommended (n. r.).

The results indicate, that the CyBi®-Well vario Nanoliter Head 384/2.5 µL allows highly precise low volume handling especially of DMSO, but also of buffer solutions in the wet-to-wet pipetting mode.

As expected, the precision data are somewhat better with DMSO solutions than with buffer solutions, but both liquid types can be handled in the wet-to-wet mode with a precision error less than 10% down to volumes as low as 50 nL (see Tab. 2 and Fig. 1).

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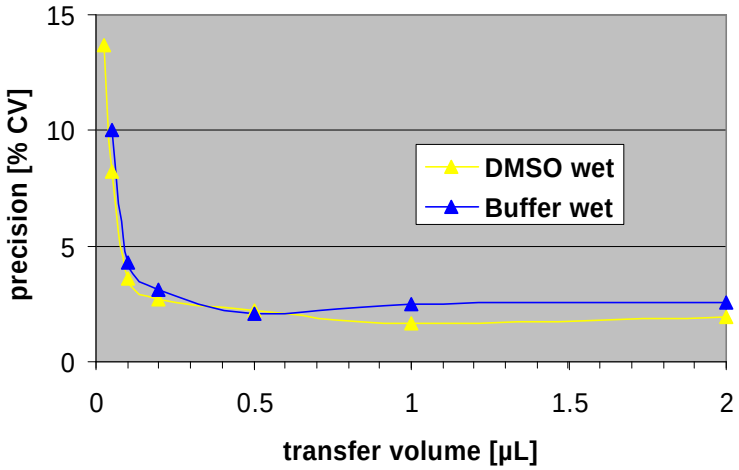

Fig.1: Precision data (fluorescence readout) obtained with the CyBi®-Well vario Nanoliter Head 384/2.5 µL, comparison of pipetting DMSO and buffer in the wet mode with 2.5 µL ceramic tips (length 41 mm).

Fig. 2 shows the precision of a selected example plate after transferring of 100 nL 120 µM fluorescein solution in DMSO with the CyBi®-Well vario Nanoliter Head 384/2.5 µL according to the method described in the sections materials and methods. The precision error of this example plate is 3.45 % CV.

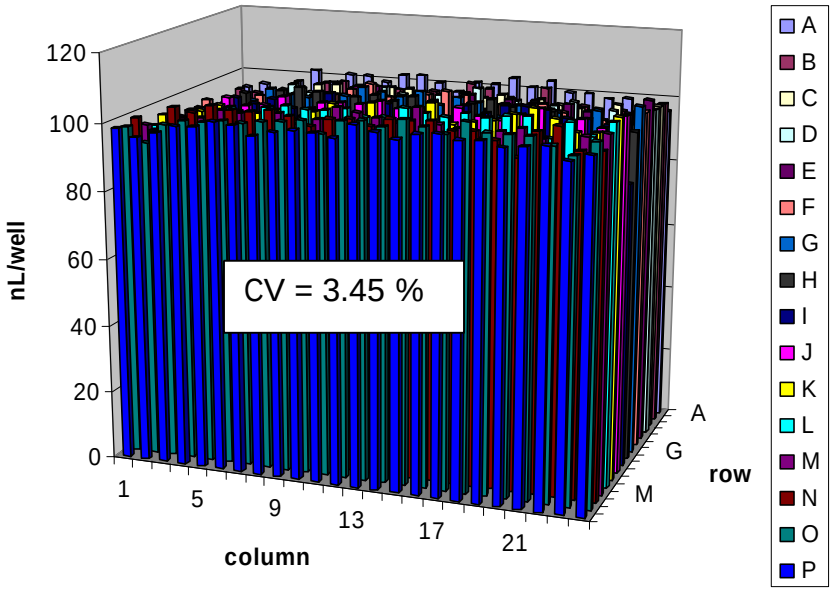

Fig.2: Selected example plate, precision data (fluorescence readout) after transfer of 100 nL 120 µM fluorescein solution in DMSO with the CyBi®-Well vario Nanoliter Head 384/2.5 µL, for experimental details see materials and methods)

Especially in the ultra-low volume range below the specification limit the influence of every experimental and methodical detail on the precision error grows up seriously.